2021

Genome-wide Association Analysis Permits Characterization of Stagonospora nodorum blotch (SNB) Resistance in Hard Winter Wheat

Rami Altameemi
South Dakota State University

GENOME-WIDE ASSOCIATION ANALYSIS PERMITS CHARACTERIZATION OF STAGONOSPORA NODORUM BLOTCH (SNB) RESISTANCE IN HARD WINTER WHEAT

BY

RAMI ALTAMEEMI

A dissertation submitted in partial fulfillment of the requirements for the

Doctor of Philosophy

Major in Plant Science

South Dakota State University

2021

DISSERTATION ACCEPTANCE PAGE

Rami Altameemi

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Jose L. Gonzalez Hernandez 6/2/2021 | 11:38 PDT

Advisor Date

Sunish Kumar Sehgal 6/4/2021 | 19:30 PDT

Advisor Date

David Wright 6/7/2021 | 06:39 PDT

Department Head Date

6/8/2021 | 08:38 CDT

Nicole Lounsbery, PhD
Director, Graduate School Date

I dedicate my dissertation work to the people who influenced me to best I can be ...

My greatest gratitude to my mom and dad for their unlimited support and love,

My twin boys for giving me such joy in the toughest times,

And my wonderful wife for her patience, kindness, and devotion...

I would like to thank my sisters and their families for believing in me. Last, I'm grateful for my parents' in-law and my sister in-law with her family for providing me with much-needed motivation and serenity.

Rami Altameemi

ACKNOWLEDGEMENTS

This work is to honor and to glorify the Almighty God, He who led to this path while guarding me and my family and guiding me throughout this mission. From the bottom of my heart, I would like to express my appreciation to all the people who shared this journey with me.

I would also like to thank my supervisor Dr. Jose Gonzalez for his energy, understanding, and help throughout my project.

I would like to give my deepest gratitude to Dr. Sunish Sehgal, my promoter and mentor My sincere thanks for all the support, his insights in this field have made this an inspiring experience for me, his thoughtful guidance, professional expertise, and advice from the very planning of this work to its execution and critical review of the manuscript and made it scientifically sound thank you for all the supporting and encouraging conversations, mails and texts that were sent to me during my dark times of this mission that gave me hope and helped me persisting and persevering, this work couldn't have come to an end without you. Dear Dr., Sunish it is a big honor for me to work with you and I am proud of being your student.

I want to give my special thanks to Dr. Shaukat Ali for providing me relevant research materials, including the cultures of plant pathogens, documents and information essential for the study; he has guided me so positively and always made me feel confident in my abilities.

I very much appreciate Dr. Brent Turnipseed and Dr. Michael Hildreth for their care and support.

I would like to express my sincere thanks to all South Dakota State University post-graduate instructors for investing their enormous efforts and precious time to shape me academically. Also, I appreciate all SDSU staff in their persistent support to accomplish this work.

My warm recognition also goes to my lab mates, Girma Ayana, Harsimardeep Gill, Jyotirmoy Halder, Ahmad Charif, and Jagdeep Singh Sidhu, Navreet Brar without your help and unconditional support this project would have not been the same.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
AM	Association mapping
ANOVA	Analysis of variance
BILs	Backcross inbred lines
CIBS	Centered identity by state
CIM	Composite interval mapping
cm	cent meter
cM	Centi Morgan
CROPS	Complexity reduction of polymorphic sequences
CS RefSeq 1.1	Chinese Spring Reference Sequence genome 1.1
DH	Doubled haploids
DNA	Deoxyribonucleic acid
ETI	Effector triggered immunity
Farm CPU	Fixed and random model circulating probability unification
GAPIT	Genomic association and prediction integrated tool
GBS	Genotyping by sequencing
GWAS	Genome wide association study
HRSW	Hard red spring wheat
HRWW	Hard red winter wheat

HWWAMP	Hard winter wheat association mapping panel
K	Kinship
ILs	Introgression lines
IWGSC	International wheat genome sequencing consortium
LD	linkage disequilibrium
LMM	Linear mixed model
LLR	Log likelihood ratios
MAF	Minimum allele frequency
Mb	Mega bytes
MCMC	Markov Chain Monte Carlo
MLE	Maximum likelihood estimation
MSG	Multiplexed shotgun genotyping
MIM	Multiple interval mapping
MTAs	Mapping trait associations
mt/ha	Metric ton per hectare
NBS-LRR	Nucleotide binding site leucine rich repeats
NEs	Necrotrophic factors
NGS	Next generation sequencing
P	Probability value
PAMP	Pathogen associated molecular pattern
PCR	Polymerase chain reaction
PTI	Pathogen triggered immunity

Ptr	*Pyrenophora tritici-repentis*
QTL	Quantitative trait loci
QTNs	Quantitative trait nucleotides
QQ	Quantile-quantile
RAD-seq	Restriction-site associated DNA sequencing
RAPD	Random amplification polymorphic DNA
RFLP	Restriction fragment length polymorphism
RILs	Recombinant inbred lines
RLKs	Receptor-like kinases
SBP	Sequence based polymorphic
SIM	Single Interval mapping
SMA	Single marker analysis
SNP	Single nucleotide polymorphism
SRWW	Soft red winter wheat
SSR	Simple sequence repeats
SWSW	Soft white spring wheat
SNB	Stagonospora Nodorum Blotch
TASSEL	Trait analysis by association, evolution and linkage
TCAP	Triticeae Coordinated Agricultural Project
USA	United States of America
USDA	United States Department of Agriculture
WAKs	Wall-associated kinases

LIST OF FIGURES

LIST OF TABLES

APPENDIX

ABSTRACT

GENOME-WIDE ASSOCIATION ANALYSIS PERMITS CHARACTERIZATION OF STAGONOSPORA NODORUM BLOTCH (SNB) RESISTANCE IN HARD WINTER WHEAT

RAMI ALTAMEEMI

2021

Stagonospora nodorum blotch (SNB) is an economically important wheat disease caused by the necrotrophic fungus *Parastagonospora nodorum*. SNB resistance in wheat is controlled by several quantitative trait loci (QTLs). Thus, the identifying of novel resistance/susceptibility QTLs is crucial for continuous improvement of the SNB resistance. Here, the hard winter wheat association mapping panel (HWWAMP) comprising accessions from breeding programs in the Great Plains region of the US, was evaluated for SNB resistance and necrotrophic effectors (NEs) sensitivity at the seedling stage. A genome-wide association study (GWAS) was performed to identify single nucleotide polymorphism (SNP) markers associated with SNB resistance and effectors sensitivity. We found seven significant associations for SNB resistance/ susceptibility distributed over chromosomes 1B, 2AL, 2DS, 4AL, 5BL, 6BS, and 7AL. Two new QTLs for SNB resistance/ susceptibility at the seedling stage were identified on chromosomes 6BS and 7AL, whereas five QTLs previously reported in diverse germplasms were validated. Allele stacking analysis at seven QTLs explained the additive and complex nature of SNB resistance. We identified accessions ('Pioneer-2180' and 'Shocker') with favorable alleles at five of the seven identified loci, exhibiting a high level of resistance

against SNB. Further, GWAS for sensitivity to NEs uncovered significant associations for SnToxA and SnTox3, co-locating with previously identified host sensitivity genes (*Tsn1* and *Snn3*). Candidate gene analysis for SNB resistance revealed 35 genes of putative interest with plant defense response-related functions. The QTLs identified and validated in this study could be easily employed in breeding programs using the associated markers to enhance the SNB resistance in hard winter wheat.

Keywords: *Triticum aestivum*, wheat, *Stagonospora nodorum blotch*, SNB, GWAS, QTL, HWWAMP, SNP markers.

CHAPTER-I

INTRODUCTION

Wheat (*Triticum aestivum* L) is an important cereal crop grown worldwide and remains a vital source for human food (FAO 2017). However, its production is continuously challenged by a number of abiotic and biotic factors (Sharma, et al. 2007b; Gupta, Chand, et al. 2018). Stagonospora nodorum blotch (SNB) caused by *Parastagonospora nodorum* (Berk) is one of the destructive fungal diseases that affects wheat and several other small grains worldwide (Dubin 1996). In susceptible cultivars, SNB symptoms are lesions that are small (1–2 mm) and water soaked in the beginning of the disease and they are usually located on the plants' lower leaves. With time these lesions have an oval shape with diffuse yellow halos (Cowger & Weisz 2013), the lesion will grow into its mature form, which characterized by having a lens shape losing their distinct yellow border. Later on, as the disease progresses, and the lesions change, now they have an ashen gray-brown center holding brown-black pepper grains (pycnidia); these lesions enlarge and unite, causing necrosis of the entire leaf. These Pycnidia are considered the diagnostic feature of this disease, which are asexual reproducing part of the fungi.

Since the fungi infect the photosynthetic apparatus as well as the glumes resulting into high yield losses and reduction in grain quality (King, Cook & Melville 1983; Eyal, Scharen, et al. 1981a ; Eyal 1981b). SNB has been reported to be common (Leath, et al. 1993) and even more common in the wheat production regions (DePauw 1995).

Currently, SNB is common in many wheat producing regions purportedly attributed to climate change and reduction in tillage or adoption of no-till practices in many wheat growing regions of the world (Liu, Faris, et al. 2004). The disease is becoming more widespread in the United States (Shaner and Buechley 1995), and during epidemics, there were losses of 30–50% (Anonymous 1995).

The most efficient and viable component of the intergraded disease management is the breeding for resistance (Crute & Pink 1996; Bartoš, et al. 2002; Duveiller, Singh & Nicol 2007; Gupta, Langridge & Mir, 2010; Khan, Tomar & Chowdhury 2010; Chowdhury, et al. 2013; Ban, et al. 2016; Vasistha, et al. 2016; Kumar, Archak, et al. 2017. Needless to say what affects the capability to develop SNB resistant cultivars are the identification of resistant genes that are responsible for SNB resistance plus the understanding of the mechanism of resistance existing in the host (Leng, et al. 2016; Osman, et al. 2016; Kumar, et al. 2017), The surge of the efficacy of breeding for disease resistance in wheat is connected with the development of molecular markers linked to disease resistance which also helps facilitate marker-assisseted selection (MAS) (Collard, et al. 2005; Gupta, Langridge & Mir 2010; Miedaner & Korzun 2012; Müller, et al. 2018). With the availability of large numbers of molecular markers (Miedaner & Korzun 2012; Korte & Farlow 2013), more efficient mapping techniques like genome-wide association studies (GWAS) have become popular for analyzing an unlimited number of traits in genetically identical materials across a wide range of environments (Gupta, Rustgi & Kulwal, 2005; Ersoz, Yu & Buckler 2007; Miedaner & Korzun 2012; Korte & Farlow 2013; Ogura & Busch 2015; Kushwaha, et al. 2017). GWAS has been used to characterize disease

resistance in many crop species: blast resistance genes in rice (Raboin, et al. 2016), maize (Xiao, et al. 2017), SB resistance in wild barley (Roy, et al. 2010), resistance to multiple leaf spot diseases of spring wheat (Gurung, et al. 2014), resistance to bacterial leaf streak and SB in spring wheat (Adhikari, et al. 2012), Fusarium head blight resistance in wheat (Arruda, et al. 2016), tan spot resistance in European winter wheat (Kollers, et al. 2014), mapping for resistance to leaf and stripe rust in winter-habit hexaploidy wheat landraces (Sun, et al. 2015).

Complex quantitative inheritance (Dubin 1996; Joshi, et al. 2004; Kumar, Tripathi & Kumar 2015) of SNB resistance in wheat has slowed the progress in breeding for SNB resistance. Many studies, using methods of both bi-parental mapping and association mapping (AM) have reported several SNB resistance QTLs on chromosome 1B, 2B, 2B, 2D, 3B, 4B, 5A, 5B, 5D, 6A, 7A and 7B (Czembor, et al. 2003; Liu, Friesen, et al. 200b; Arsenuik, et al. 2004; Gonzalez-Hernandez, et al. 2009; Friesen, et al. 2009; Aguilar, et al. 2005; Shankar, et al. 2008; Francki, et al. 2011; Schnurbusch, et al. 2003; Uphaus, et al. 2007). These QTLs confer resistance to either seedling or adult plant resistance to SNB.

Most of these studies have been focused on hard spring wheat, and relatively few studies characterized SNB resistance in hard winter wheat germplasm. Our ability to deploy and develop SNB resistant winter wheat cultivars depends on the identification of resistant QTLs responsible for the traits.

Objectives

1. Identify and evaluate the genetic basis of resistance against SNB.
2. Identify SNP markers associated with sensitivity to SnToxA, SnTox1, and SnTox3.
3. Identify candidate genes underlying MTAs for SNB resistance.

CHAPTER-II

LITERATURE REVIEW

2.1 Biology of bread wheat

Wheat (*Triticum aestivum* L) is one the major cereal crops grown globally as a source of carbohydrates. Other crops grown as a source of carbohydrates include maize (*Zea mays* L.), rice (*Oryza sativa* L.), sorghum (*Sorghum bicolor* L.), barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.). Wheat belongs to the grass family *Poaceae*, which has more than 10,000 species generally with global distributions. The evolution of domesticated wheat has involved interspecific crosses and chromosome doubling. For example, the first two cultivated wheat species, wild Emmer (*Triticum dicoccoides*) and wild Einkorn (*Triticum urartu*), both had seven chromosomes (n-7). Wild emmer is a natural hybrid between *T. urartu* and unknown goatgrass species giving rise to a fertile tetraploid progeny with seven chromosomes from each parent that had underwent spontaneous chromosome doubling (2n = 28). The current bread wheat species (*Triticum aestivum*) is a cross between emmer and goatgrass (*Aegilops squarrosa*) followed by chromosomes doubling formed a fertile hexaploid (2n = 42) progeny. The family *Poaceae* are monocotyledonous, with parallel veins on the leaves, and with flowers that are pollinated by wind. Wheat has long slender leaves and the stems are hollow in many cultivars. There is variation in the number of leaves and plant height. The inflorescences comprise varying numbers of minute flowers ranging from 20 to 100 that are borne in clusters ranging from two to six in structures known as spikelet. Each spikelet houses two or three

seeds (i.e., grains), which develop from embryos after the flowers are successfully pollinated and fertilized. Wheat can grow in a range climatic regions and soils; temperate regions that receive 30cm to 90cm (12 to 36 inches) during the growing season are best for wheat production.

new paragraph There are several methods used to classify wheat. They are classified as winter- or spring-type based on the season they are sown. Winter wheat requires vernalization treatment for it to flower, hence it is planted in fall. There is no vernalization treatment required for spring wheat, hence planting is done in the spring. Classification based on the color of its grain divides wheat varieties into either be white or red. Another classification is based on the hardness or softness of the endosperm divides them into either hard or soft wheat. Combinations of the aforementioned classification methods are used to describe the various cultivars. For instance, the cultivar classified as "Soft White Spring" wheat is an important cereal crop grown worldwide and remains a vital source for human food (FAOSTAT, 2017).

new paragraph During2015-2116, wheat ranked first among cereals in total production (USDA, 2017), and was cultivated on approximately 225.07 million hectares of land that yielded 736.98 metric tons worldwide. During the same time, the USA produced 62.86 metric tons of wheat from which 62% consisted of winter wheat. The current productivity of the USA and the world respectively reached 3.4 mt/h and 3.12 mt/h, and it has increases 0.9% every year. Yet, the projected demand needed to feed the 9 billion human population projected for 2050 will require that productivity of wheat will need to increase by a rate of 2.4% per year (Ray *et al*., 2013).

2.2 Use of bread wheat

There are three types of wheat used in baking and food production. Durum wheat produce high gluten protein compared to the “Hard” and “Soft” wheats. They have the hardest endosperm and are used in producing semolina that is transformed into macaroni and pastas. “Hard” wheat produces higher levels of gluten proteins than “Soft” wheat. Hard Red Winter (HRW) and Hard Red Spring (HRS) wheats are used for making bread flours and all-purpose flours. Finally, Soft Red Winter (SRW) and Soft White Spring (SWS) wheats are used for making cake and pastry flours.

2.3 Global Wheat production and its importance

Among the cereal crops produced globally, wheat is one of the cereals grown on a wide area and consumed by a greater population (USDA, 2017). Approximately 35% of global population relies on wheat as its staple food crop (FAO, 2013). The area under cultivation and its yields in the 2015-2016 seasons were estimated at 225 million hectares and 737 million metric tons respectively. The world leading producers of wheat are the European Union, China, India, Russia and USA in that order (USDA, 2017). Despite China being the largest producer of wheat, it is also a net importer because of it has the highest human population and because its socio-economic living standards have pushed up consumption demands for wheat-related food items.

2.4 Wheat production in USA

In the USA, the field crop area is dominated by corn, soybeans and wheat, and occurs primarily in the Midwest region in the USA Statistics from 2014-2016 indicate that Kansas (KS), North Dakota (ND), Montana (MN), Washington State (WA),

Oklahoma (OK), South Dakota (SD), Colorado (CO), Idaho (ID), Texas (TX) and Minnesota (MN) were states with the highest production of wheat (USDA-NASS, 2017). Total wheat produced in the USA among all the categories was estimated to be 63 million metric tons from a total area of 17,761, 840 hectares (USDA-NASS, 2017). Wheat production created a variety of business opportunities in the value chain, hence improving livelihoods to the farming community and other service providers related to the wheat industry.

2.5 Tools used for breeding for resistance against diseases

Plant pathogens and their hosts have always coexisted in nature (Thrall & Burdon, 2002), and there is an arms continual race between pathogens and hosts. Flor (1942) described the coexistence of the host-pathogen relationship, using gene for gene theory developed through studies conducted in oats infected with a fungus. The studies revealed that the fungi were able to overcome resistance strategies developed by hosts if they were given continuous exposure to the pathogen. Thus, the selection pressure by the pathogen overcomes the host resistance with the passage of time. Flor (1942) proposed that for any virulence gene in the pathogen there was a corresponding gene for resistance in the host. In an interaction between virulence and a virulence gene, a disease reaction occurs, but if there is no incompatible reaction, there is no disease reaction. However, the interaction between a pathogen and its host may not result in disease in the absence of a favorable environment. A disease reaction will occur only if the three components are present and favorable (Grulke, 2011). The basis for developing cultivars that are resistant to pathogens relies on the theory that Flor (1942) developed. However, this theory assumes

that a single gene in the pathogen interacts with a single gene in the host, which is not the case in many instances. Multiple genes function as a unit in conferring resistance in a host, hence the hypothesis developed by Flor (1942) may have limited applications.

An understanding of the host range for pathogens is crucial in host-pathogen management. Some pathogens derive nutrients, growth and reproductive activities in a wide range of hosts; these are known as polyphagous pathogens. On the contrary, monophagous pathogens derive their nutrients, growth and reproductive activities in few very closely related hosts. Therefore, monophagous pathogens function as specialists. Development of sustainable, environmentally friendly and economical approaches is vital in managing diseases in the presence of arms-race between the pathogens and hosts. Using host resistance as an approach in disease management, has proved to be an effective strategy in controlling many diseases caused by a variety of bacterial, fungal, viral and insect pests (Agrios, 1988;Bradshaw, 2016; Fry, 1982; Van Loon, 1997).

2.6 Mendelian strategy

The precise record keeping, analysis, interpretation and publication of Gregor Mendel's studies on peas, provided the basis for other scientist's work on the inheritance of traits including the disease resistance work on flax rust disease by Flor (1955, 1956). Flor unveiled the gene-gene theory that deciphered the interaction between the pathogen and host. The theory underscores the reliance on the single dominant resistant (*R*) gene in a host that interacts with a specific protein, which is a recessive virulent gene (*avr*) in the pathogen. Interaction of the *R* and *avr* genes results in an incompatibility reaction that is specific and that triggers a cascade of events in the host culminating into activation of

defense machinery in the host. This mechanism provides its host with a defense against pathogens. Through Flor's studies, they were able to observe the fungi losing the *avr* gene, hence defining it as incompatible; implying it as ability to parasitize. Advances in biology, molecular biology, biochemistry, computing and statistical tools led to the cloning of the *Avr* gene from *Pseudomonas syringae* (Staskawicz, et al., 1990) and first *R* gene, *HM1* (Johal & Briggs, 1992). Currently, many studies have discovered several hosts related proteins with pathogen virulence targets (Rooney et al., 2005; Mackney, et al., 2003). Another study revealed the interaction of *R* gene, Nucleotide Binding Site Leucine Rich-Repeats (NBS-LRR) protein domain that is crucial in host defense mechanisms (Kugler, 2013; Bekhaldir, et al., 2004).

2.7 Vertical and horizontal disease resistance

Disease resistance can be introduced into new cultivar lines as vertical or horizontal resistance. Vertical resistance is also known as qualitative resistance, which is discreet and controlled by a minor gene (monogenic). Its expression is not affected by the environment and is pathogen race specific fitting the gene-gene theory. Furthermore, qualitative disease resistance is simple for breeding because of its simplicity in inheritance, measurement and not being affected by environment. On the other hand, it is not durable compared to horizontal resistance (Bhadauria & Propescu, 2017; French, et al, 2016; Flor H. , 1942;Krattinger & Keller, 2016).

Horizontal resistance is also known as quantitative resistance since it is under the control of many genes hence polygenic. It is usually expressed in the adult phase of the crop growth and development. (Corwin & Kliebenstein, 2017). Polygenic gene inheritance

implies that a single trait that is controlled by at least more than two genes (Bhadauria & Popescu, 2017). Traits with horizontal inheritance are caused by segregation of multiple genes, each contributing a minor effect on the trait, hence having a continuous variation on the trait in the population. Environment factors affects the expression of the traits under polygenic control hence exhibiting a normal distribution continuous range of disease reaction. The gene-to-gene hypothesis is not applicable for quantitative traits. Horizontal resistance is classified as durable and stable race nonspecific to pathogens since many minor genes are involved in disease resistance (Bhadauria & Popescu, 2017). Horizontal resistance does not offer immunity to the host but slows down the development of the disease in a population. Plant breeders use horizontal resistance in developing lines that possess multiple genes conferring resistance against a range of pathotypes, the cultivars being grown in a wider area, ultimately remaining effective for longer periods. These cultivars usually do not possess hypersensitive inducing genes (Mundit, 2014; Bariana et al., 2001; Johnson, 1981, Johnson & Law, 1975). Selection procedures for quantitative resistance are painstaking to breeders as opposed to qualitative resistance. Continuous variation in the expression of disease reactions in the population and environmental effects in the expression of phenotypes poses selection procedures unlike in qualitative resistance where phenotype expression is absolute (Bhadauria & Popescu, 2017). Furthermore, a pathogen's population is not classified on the basis of race differential, but on the basis of individuals within the population that may exhibit variability on aggressiveness and virulence traits. Heritability is usually low in quantitative resistance, since environmental factors are among the factors that affects

its transmission from parent to offspring in traits that are controlled by multiple genes (Bhadauria & Popescu, 2017; French et al,, 2016; Krattinger & Keller, 2016).

2.8 Genetic markers

Genetic markers are synonymously known as molecular markers in the field of genetics. They are fragments of a known sequence of deoxyribonucleic acid (DNA) that is associated with a known trait located in a genome of an organism. Use of molecular/genetic markers significantly improves the selection and fast tracking for locating genes and thereby individuals possessing genes that control targeted traits such as yield, agronomic traits, resistant to pests and diseases as quality traits in plants (Dreisigacker et al., 2016).

Application of molecular markers in life sciences has undergone tremendous improvement over time, especially with the discovery and adoption of the polymerase chain reaction (PCR) and high throughput sequencing technologies. During early stages molecular marker technology, low throughput, gel and hybridization-based markers, like restriction fragment length polymorphism (RFLP) not PCR-based, while random amplification polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), were the markers of the utilized in many life sciences and were medium throughput (Semagn, Bjostad, & Ndjiondjop, 2006). These markers were used in fingerprinting and germplasm characterization of different crops without prior genomic information. Nevertheless, use of these markers had many challenges that include large quantity of DNA sample in case of RFLP, labeling, DNA

fragmentation, electrophoresis, blotting, hybridization, washing and finally band imaging. The aforementioned processes were time consuming, slow and cumbersome, and would take several weeks to complete the process. Improvements in molecular biology, bioinformatics and statistical computing, led to the introduction of other markers for breeders. Markers such as SSR and single nucleotide polymorphism (SNP) were adopted in many laboratories replacing the first line of markers that had low throughput technology. The discovery of sequencing technologies led to rapid and global adoption of SNP markers that are generated by point mutations in the genome (Wang et al., 2014). The popularity of SNP markers in genetic studies is attributed to their genome-wide abundance, robustness in constructing highly saturated genetic maps, and capability of capturing variability in many parents within a limited time span (Korte & Farlow, 2013; Miedaner et al., 2012).

2.9 Next-generation sequencing (NGS)

Next generation sequencing (NGS) synonymously known as high-throughput sequencing, encompasses all modern sequencing technologies that include Illumina (Solexa) sequencing, Roche 454 sequencing, Ion torrent, Proton/PMG sequencing and SOLiD sequencing. NGS has revolutionized genomic studies due to its low operational cost (purchase cost is high for the equipment and chemicals), nevertheless, the benefits outweigh the costs when considering accuracy and automation associated with the technology (Mardis & Elaine, 2008).

The protocol for NGS involves DNA template preparation, library preparation, ligating adapters to randomly sheared genomic DNA fragments, genome sequencing and analysis

of the sequences produced (Metzker, 2010). There are two methods used in partial genome library preparation in NGS platforms. The first method uses restriction enzymes and is known as complexity reduced representation library. Its pros include its simplicity and quickness to organize, its high specificity and reproducibility; it also avoids repetitive segments and can reach the regions of genome not accessible to sequence capture methods. The restriction enzymes commonly used with this library include reduced-representation libraries (Gore, et al., 2009), restriction-site associated DNA sequencing (RAD-seq) (Rowe et al., 2011), complexity reduction of polymorphic sequences (CROPS) (Mammadov, et al., 2010), sequence based polymorphic marker technology (SBP) (Sahu et al,, 2012), low multiplexed shotgun genotyping (MSG) (Andolffatto, et al., 2011) and genotyping by sequencing (GBS) (Elshire, et al., 2011). The second method used in library construction and representation does not use restriction digestion enzymes.

2.10 Genotype-by-sequencing (GBS)

Genotype-by-sequencing (GBS) is a molecular tool-box that can be used for molecular genetic studies ranging from a single gene marker to the whole genome profiling of organisms (Poland & Rife, 2012). The protocol is highly multiplexed for constructing reduced representation libraries in Illumina NGS platforms. Its popularity has been high because it bypasses the whole marker assay development phase by simultaneously detecting and scoring SNPs (Elshire, et al., 2011).

The protocol for GBS involves sample preparation: DNA extraction, quantification, restriction digestion with GBS enzymes and adapter ligation and legit cleaning. The stage

after ligation involves pooling DNA samples, PCR amplification with primers that are specific to each adapter. The DNA sequences developed in FASTQ format file are downloaded then and subjected to culling. SNP culling involves collapsing the reads, contigs assembly, aligning the reads to the contigs, summarizing the alignments made and ultimately culling the SNPs relative to one another or to a specific reference genome (Wallace & Mitchell, 2017).

2.11 Gene mapping

2.11.1 Linkage mapping

Linkage maps are genetic maps of species that show the position of the known genes or genetic markers relative to each other in the form of recombination frequencies as opposed to the physical distance along each chromosome (Hyten & Lee, 2016; Somers et al., 2004; Collard et al., 2005). Linkage maps show the relative positions of molecular markers along chromosomes, which are determined by recombination frequencies during crossover events of homologous chromosomes in the meiosis process. The quantitative trait loci and Mendelian gene systems are subject for evaluation. Furthermore, the maps determine the recombination frequencies of temporary and permanent populations from crosses of two or pure lines; using polymorphic markers to genotype, constructing a linkage map and ultimately analyzing the association existing between genetic markers and important genes determining a character (Collard et al., 2005).

Identification of two or more parents with contrasting phenotypic variation on the trait of interest, then forming a bi-parental population is the first phase in linkage mapping

(Anderson et al., 1993). Choice on the population to use for creating a linkage map depends on the objectives of the study. Populations can be created using F2:3, recombinant inbred lines (RILs), double-haploids (DH), introgression lines (ILs), and backcross inbred lines (BILs) (Mohan, et al., 1997; Collard et al., 2005). Use of F3 phenotypic values for a trait under study is recommended in situations where species have inherently low heritability for a trait under evaluation and usually produce less seed. Furthermore, the power of the QTL mapping may be unreliable, and the usage of the population may be limited in situations when F2 seed is used for the mapping exercise. Thus, F2 seed is still undergoing segregation and recombination during early stages of a generation, unlike the later phases of the RILs (Zeng, 1993; Lander & Bostein, 1989).

2.11.2 QTL mapping models

Improvements in statistical computing and the development of powerful algorithms and software have facilitated release of tool kits that assist analyzing linkage maps. Currently, there are four models that are used in linkage maps, including: 1) single marker analysis (SIMA), 2) simple interval mapping (SIM) for single marker analysis, 3) multiple interval mapping (MIM) and 4) composite interval mapping (CIM) (Collard et al., 2005). These models are utilized to establish an association between the markers and the traits of interest. Statistical procedures such as analysis of variance (ANOVA), t-test, regression analysis, maximum likelihood estimation, and log likelihood ratios are used for hypothesis testing if a given class of genotype is different in phenotype for a suggested marker or a set of molecular markers.

Single marker analysis is applicable in situations where there is no requirement to develop a complete linkage map. Thus, it is useful in analyzing QTLs using unlinked marker data. It is the easiest method compared to other methods in QTL mapping. There are some disadvantages with using the method. One of the shortfalls, is that it increases the effects of underestimating the effects of the analysis. This may arise due to recombination between markers and the increase in the number of single marker comparisons ultimately increasing the false positives (type 1 error rate). The second weakness with the SMA model is they cannot identify the markers that are associated with one or more QTLs. Furthermore, SMA models cannot give separate estimates of QTLs and separate QTL estimates for sites. Other studies have suggested increasing the number of molecular markers to offset the shortfalls, but the reliability of the model is limited (Collard et al., 2005).

The interval mapping model estimates the location of a putative QTL associated with the character under study in the genome in intervals between flanking markers. Statistical procedures like regression analysis and maximum likelihood are used to approximate the QTL within the flanking markers. Two methods within interval mapping were designed to run mapping, namely simple interval mapping (SIM) and composite interval mapping (CIM). SIM analyzes the linkage disequilibrium between a potential QTL and the flanking markers. The challenge with using SIM is that it analyzes for the presence of the QTL between the intervals of marker loci irrespective of any factor outside the interval that may influence the results (Collard et al.,, 2005). In general SIM models are more robust compared to SMA.

Use of composite interval mapping takes care of the shortfalls identified in SIM models. Thus, CIM minimizes the confounding effects of other QTLs positioned outside the flanking markers. Any QTL effects outside the range of the two flanking markers are regarded as background variation. CIM incorporates cofactors, which are a set of markers to reduce the effects of background variation. The cofactor markers comprise the linked and unlinked markers that are highly associated with the trait and they may be positioned anywhere in the genome. Incorporation of the cofactor markers in the model assists in minimizing the confounding effects of other QTLs. The inclusion of the cofactors in the model makes it a robust tool in handling multiple QTLs in the analysis unlike SMA and SIM (Collard et al., 2005).

2.11.3 Association mapping

Association mapping (AM) is a procedure of mapping QTLs to uncover association between a phenotype and a genotype. Association mapping is also synonymously known as linkage disequilibrium mapping (Gupta et al., 2005). Ersoz et al. (2007) defined linkage disequilibrium (LD) as the non-random co-segregation of alleles at two or more loci. Factors like natural selection, genetic drift, mutations and recombination maintains LD in the species genome. Haplotype blocks are formed by non-overlapping sets of loci with strong LD. The pedigree with haplotypes exhibiting excess transmission of the same alleles to progenies (Gupta et al., 2005). The prowess of association mapping tool-box hinges on the exploitation of all previous recombination and mutations events in the population. The objective of association mapping seeks to identify inter-individual

genetic variants with SNPs that show highest association to the characters under study; the SNP should be the causal gene or highly correlated to the causal gene.

An application of association mapping includes identifying genetic variants in documented genomic regions of a species, which is a typical approach used in candidate gene. Other studies use association mapping in examining representatives of SNP variants across the entire genome, an approach used in genome wide association studies (Erson et al., 2007).

An advantage of association mapping over other models like linkage mapping is its capability to carter for many parents available in the genetic diversity studies. This is not the case with the linkage mapping protocol that requires few parents. Using many parents increases the chances of discovering many alleles concurrently creating an opportunity of finding many minor genes controlling quantitative traits. A second advantage of using association mapping as a protocol is that it reduces time and costs resources in the creation of recombinant lines for evaluation. Furthermore, association mapping may utilize past season's phenotype data in its evaluation. Nevertheless, challenges associated with kinship may exist in the germplasm and the structure of the population. Therefore, there is a need to exercise precautionary measure when using association mapping in the study (Ersoz et al., 2007; Korte & Farlow, 2013).

2.12 Challenges threatening wheat production

2.12.1 Major factors limiting wheat production

There is a big gap between the wheat yields levels reported under field experimentation versus the yields typically attained in farmer's plots. Factors influencing the gap are collectively classified into biological, technological, ecological, socio-economical and a combination of different levels of interactions between and among the factors. Climate change is projected to have an impact on global wheat production in future (Asseng, et al., 2015; Placeholder3; Ray et al., 2015). Pests and diseases are the main biological factors that affects grain yield of wheat and other crops, depending on the stage of plant development when the damage occurs to the crop. Some diseases have been reported to cause 100 percentage damage when the field is infected with a pathogen. In general, most diseases are caused by bacteria, fungi and/or viruses. They infect different plant parts, use different mechanisms causing infection and use different dispersal mechanisms. Among biological factors, diseases and pests are known to reduce grain yields that remain to be economically important. Some crop diseases are already well established in regions while the importance of others is becoming more important as various factors have led to their emergences. Closing the yield gap caused by a particular wheat disease requires research to improve the development of resistant varieties and hybrids, improvements in biotechnology techniques, a and a better understanding host physiology and their interaction with damaging pests (Tester & Langridge, 2010).

2.12.2 Biology of the pathogen causing SNB

Septoria nodorum blotch (SNB) is a fungal disease caused by *Parastagonospora* (Quaedvlieg, et al., 2013; Solomon et al., 2006). The causal agent is also known as *Stagonospora* or *Phaesphaeria* (Quaedvlieg, et al., 2013). Multiple genes (Abeysekera et al, 2009) govern the infection process through the interaction of fungal necrotrophic effectors (NEs) with the host dominant sensitivity gene products (Faris & Zhang, 2010; Tan et al., 2010). Interactions that are compatible produce host tissue necrosis in a host which facilitates pathogen infection, and these necrotrophic pathogens derive their nutrition for growth and reproduction from the dead plant cells or tissues achieved by the pathogen's aggressive and wide-ranging virulence traits. They disarm host immune defense mechanisms through use of toxins or enzymes that kill host tissues (Horbach et al., 2011; Mengiste, 2012). The first effector gene identified in *P. nodorum* was *ToxA* and there is strong evidence that *ToxA* was first identified in the tan spot fungus, *Pyrenophora tritici-repentis* (*Ptr*), Friesen et al. (2006) provided evidence on a hypothesis that there was a horizontal gene transfer from *P. nodorum* to *PtrToxA*. Cultivars that carry *Tsn1* gene are susceptible to *P. nodorum* since the *ToxA* encodes a small, secreted protein that induces necrosis and facilitates infection process (Tan et al., 2012). 5B chromosomes carries the *Tsn1* gene that encodes a distinct and specific protein NBS-LRR along with another protein with a kinase domain (Faris et al., 2010). A study by Liu et al. (2009) demonstrated that *SnTox3* was capable of secreting a small protein. Another study revealed that the sensitivity of *SnTox3* is affected by *Snn3-B1* and *Snn3-D* genes. Snn3-B1 is located on chromosomes 5B while Snn3-D1 is located on chromosome 5D (Zhang,

et al., 2011). Several studies (Friesen et al., 2006; Liu et al., 2009; McDonald et al., 2013; Oliver et al., 2012) demonstrated the roles played by the *Snn3-B1* and *Snn3-D1*genes on the virulence of SNB disease.

2.12.3 Strategies used to manage the disease

Several strategies have been developed and promoted for managing SNB. The approaches include improving host genetics, fungicide applications and modifying agronomic practices such as crop rotation (Francki, 2013). Improving host genetics is a safer and more environmentally friendly approach to managing SNB compared to chemical control methods. The discovery of many QTLs conferring quantitative and qualitative resistance offers an opportunity in speeding up gene introgression in many lines globally in managing the disease (Francki, 2013). Studies by Friesen, et al. (2006) Liu, et al. (2012) and Gurung et al.(2014) reported the existence of genotype-specific effector proteins on ToxA, Tox1 and Tox3 that may affect the development of new lines and deployment to the farming community in wheat growing ecologies. On the contrary, the existence of genotype-effector protein has been commercially exploited in producing an assay for screening lines sensitive to the assay (Vleeshouwers & Oliver, 2014). The technique involves applying the assay to the leaves through needleless syringe infiltration, then observe reactions after a specified time frame. All lines that are not sensitive to the assay are advanced in the breeding program and become potential candidates for release to the farming community. The methodology demonstrates how discoveries in molecular plant-pathogen interactions may fast-track disease management practices.

There are several fungicides that have been developed and deployed to the farming community to effectively control SNB. Use of chemical control, on the other hand, comes with many caveats. For example, the pathogen can develop resistance to the chemicals if there is continuous use of the same fungicide over a period of time. Blixt et al. (2009) reported resistance in some isolates of SNB to the Qol fungicide azoxystrobin in a Scandinavian study.

Reference

Abdullah, S., & al., e. (2017). Characterization of Pyrenophora tritici-repentis (Tan spot of wheat) races in Baltic states and Romania. *Plant Pathol. J., 33*, 133-139.

Abeysekera, N., Friesen, T., Keller, B., & Faris, J. (2009). Identification and characterization of a novel host-toxin interaction in the wheat-Stagonospora nodorum pathosystem. *Theor. Appl. Genet., 120*, 117-126.

Adhikari, T. B., Gurung, S., Hansen, J. M., & al., e. (2012). Association mapping of quantitative trait loci in spring wheat landraces conferring resistance to bacterial leaf streak and spot blotch. *Plant Genome, 5*, 1-16.

Adhikari, T., Jackson, E., Gurung, S., Hansen, J., & al., e. (2011). Association mapping of quantitative resistance to Phaeosphaeria nodorum in spring wheat landraces from the USDA national small grains collection. *Phytopathology, 101*, 1301-1310.

Agrios, G. (1988). *Plant Pathology* (Vol. 3rd Ed.). New York: Academic Press Inc.

Aguilar, V., Stamp, P., Winzeler, M., & al., e. (2005). Inheritance of field resistance to Stagnospora nodorum leaf and glume blotch and correlations with other morphological traits in hexaploid wheat (Triticum aestivum L.). *Theor. Appl. Genet., 111*, 325-336.

Ahmad, I., Ali, N., & Ahmad, H. (2017). Association mapping of root traits for drought tolerance in bread wheat:. In W. a. R (Ed.), *Wheat Improvement, Management and Utilization* (pp. 39-57). London: InTech.

Amalaraj, A., Luang, S., Kumar, M. Y., & al, e. (2016). Change of function of the wheat stress-responsive transcriptional repressor TaRAP2.1L by repressor motif modification . *Plant Biotechnol. J., 14*, 820-832.

Anderson, J., Churchill, G., Autrique, J., Tanksley, S., & Sorrels, M. (1993). Optimizing parental selection for genetic linkage maps. *Genome, 36*, 181-186.

Angus, J., Gardner, P., Kirkegaard, J., & Desmarchelier, J. (1994). Biofumigation: isothiocyanates released from Brassica roots inhibit growth of the take-all fungus . *Plant and Soil, 162*, 107-112.

Anonymous. (1995). *Losses in Agriculture: Agriculture Handbook No. 291.* Washington DC: United States Department of Agriculture.

Anonymous. (1995). *Losses in Agriculture: Agriculture Handbook No. 291.* Washington DC: United States Department of Agriculture.

Araus, J. L., Slafer, G. A., Royo, C., & al., e. (2008). Breeding for yield potential and stress adaptation in cereals. *Crit. Rev. Plant Sci., 27*, 377-412.

Arruda, M. P., Brown, P., Brown-Guedira, G., & al., e. (2016). Genome-wide association mapping of Fusarium head blight resistance in wheat using genotyping-by-sequencing. *Plant Genome, 9*, 1-14.

Arseniuk, E. e. (2004). QTL controlling partial resistance to Stagnospora nodorum leaf blotch in winter wheat cultivar Alba. *Euphytica, 137*, 225-231.

Arsenuik, E., Czembor, P. C., Czaplicki, A., & al., e. (2004). QTL controlling partial resistance to Stagnospora nodorum leaf blotch in winter wheat cultivar Alba. *Euphytica, 137*, 225-231.

Asseng, S., Ewert, F., Martre, P., Rotter, R., Lobell, D., Cammarano, D., & Reynolds, M. (2015). Rising temperatures reduced global wheat production. *Nature Climate Change, 5*, 143-147.

Axtell, M., Chisholm, S., Dahlbeck, D., & Staskawicz, B. (n.d.). Genetic and molecular evidence that Pseudomonas syringae type III effector protein AvrRpt2 is a cysteine protease . *Molecular Mricrobiology, 49*, 1537-1546.

Ayalew, H., Liu, H., & Yan, G. (2017). Identification and validation of root length QTLs for water stress resistance in hexaploid wheat (Triticum aestivum L.). *Euphytica, 213*, 126.

Ayana, G., & al., e. (2018). Genome-wide association study for spot blotch resistance in hard winter wheat. *Front. Plant Sci., 9*.

Ballance, G., Lamari, L., & Bernier, C. (1989). Purification and characterization of a host-selective necrosis toxin from Pyrenophora tritici-repentis. *Physiol. Mol. Plant Pathol., 35*, 203-213.

Ban, T., Singh, V., Singh, G., & al, e. (2016). Phenotyping at hot spots and tagging of QTLs conferring spot blotch resistance in bread wheat. *Mol. Biol. Rep. , 43*, 1293-1303.

Bariana, H., Hayden, N., & Ahmad, e. a. (2001). Mapping durable adult plant and seedling resistance to stripe and stem rust disease in wheat. *Australian J. of Agric. Res., 52*, 1247-1255.

Bartoš, P., Šip, V., Chrpová, J., & al., e. (2002). Achievements and prospects of wheat breeding for disease resistance. *Czech J. Genet. Plant Breed. , 38*, 16-28.

Bengough, A. G., Gordon, D. C., Al-Menaie, H., & al., e. (2004). Gel observation chamber for rapid screening of root traits in cereal seedlings . *Plant Soil, 262*, 63-70.

Bertin, C., Yang, X., & Weston, L. (2003). The role of root exudates and allelochemicals in the rhizosphere . *Plant and Soil, 256*, 67-83.

Bhadauria, V., & Popescu, L. (2017). Quantitative genetics of disease resistance in wheat. *Current Issues in Molecular Biology*(27), 105.

Bhadauria, V., & Popescu, L. (2017). Quantitative genetics of disease resistance in wheat. *Current Issues in Molecular Biology, 27*, 105.

Bhadauria, V., & Popescu, L. (2017). Quantitative genetics of disease resistance in wheat. *Current Issues in Molecular Biology, 27*, 105.

Bhadauria, V., & Popescu, L. (2017). Quantitative genetics of disease resistance in wheat . *Current Issues in Molecular Biology, 27*, 105.

Bhadauria, V., & Propescu, L. (2017). Quantitative genetics. of disease resistance in wheat. *Current Issues in Molecular Biology, 27*, 105.

Bhathal, J., Loughman, R., & Speijers, J. (2003). Yield reduction in wheat in relation to leaf disease from yellow (tan) spot and septoria nodorum blotch. *Eur. J. Plant Pathol., 109*, 435-443.

Blixt, E., Djurie, A., Yuen, J., & Olson, A. (2009). Fungicide sensitivity in Swedish isolates of Phaeosphaeria nodorum. *Plant Pathology, 58*, 655-664.

Bostwick, D., Ohm, H., & Shaner, G. (1993). Inheritance of resistance of Septoria glume blotch resistance in wheat. *Crop Sci., 33*, 439-443.

Bradbury, P., & al., e. (2007). TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics , 23*, 2633-2635.

Bradshaw, J. (2016). *Plant breeding: Past, present and Future.* New York: Springer.

Bruno, H., & Nelson, L. (1990). Partial resistance to Septoria glume blotch analyzed in winter wheat seedlings . *Crop Sci., 30*, 54-59.

Buckler, E. S., Holland, J. B., Bradbury, P. J., & al, e. (2009). The genetic architecture of maize flowering time. *Science, 325*, 714-718.

Carter, T., DeSouza, P., & Purcell, L. (1999). Recent advances in breeding for drought and aluminium resistance in soybean. *Proceedings of the World Soybean Research Conference VI*, (pp. 106-125). Champaign, IL.

Cavanagh, C., & al., e. (2013). Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars . *Proc. Natl. Acad. Sci.*, doi:10.1073/pnas.1217133110.

Chan, M., & Close, R. (1987). Aphanomyces root rot of peas3. Control by use cruciferous ammendments . *New Zealand Journal of Agricultural Research, 30*, 225-233.

Chang, H., Chen, D., Kam, J., & al, e. (2016). Abiotic stress upregulated TaZFP34 represses the expression of type-B response regulator and SHY2 genes and enhances root to shoot ratio in wheat. *Plant Sci., 252*, 88-102.

Cheema, Z. A., Khaliq, A., & Saeed, S. (2004). Weed control in maize (Zea mays L.) through sorghum allelopathy. *Journal of Sustainable Agriculture, 23*, 73-86.

Chowdhury, A. K., Singh, G., Tyagi, B. S., & al., e. (2013). Spot blotch disease of wheat–a new thrust area for sustaining productivity. *J. Wheat Res., 5*, 1–11.

Christopher, J. L., Christopher, M., Jennings, R., & al., e. (2013). QTL for root angle and number in a population developed from bread wheats (Triticum aestivum) with contrasting adaptation to water-limited environments. *Theor. Appl. Genet., 26*, 1563-74.

Christopher, J. T., Manschadi, A. M., Hammer, G. L., & al., e. (2008). Development and physiological traits associated with high yield and stay-green phenotype in wheat. *Aust. J. Agric. Res., 59*, 354-64.

Ciuffetti, L., Tuori, R., & Gaventa, J. (1997). Single gene encodes a selective toxin causal to the development of tan spot of wheat. *Plant Cell, 9*, 135-144.

Collard, B. C., Jahufer, M. Z., Brouwer, J. B., & al., e. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker assisted selection for crop improvement: the basic concepts. *Euphytica, 142*, 169-196.

Collard, B., Jahufer, M., Brouwer, J., & Pang, E. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker assisted selection for crop improvement: the basic concepts. *Euphytica, 142*, 169-196.

Collard, B., Jahufer, M., Brouwer, J., & Pang, E. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica, 142*, 169-196.

Collard, B., Jahufer, M., Brouwer, J., & Pang, E. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica, 142*, 169-196.

Collard, B., Jahufer, M., Brouwer, J., & Pang, E. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica, 142*, 169-196.

Collard, B., Jahufer, M., Brouwer, J., & Pang, E. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts . *Euphytica, 142*, 169-196.

Collard, B., Jahufer, M., Brouwer, J., & Pang, E. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts . *Euphytica, 142*, 169-196.

Collard, B., Jahufer, M., Brouwer, J., & Pang, E. (2005). An introduction to markers, quantitative trait loci (QTL) mapping, and marker-assisted selection for crop improvement: the basic concepts. *Euphytica, 142*, 169-196.

Corwin, J., & Kliebenstein, D. (2017). Quantitative resistance: more than just perception of a pathogen. *The Plant Cell, 29*, 655-665.

Cowger, C., & Weisz, R. (2013). *Small grain disease management. In: Small grain production guide.* North Carolina Cooperative Extension Services.

Cowger, C., & Weisz, R. (2013). *Small grain disease management. In: Small grain production guide. .* North Carolina Cooperative Extension Services.

Cowger, C., Ward, B., Brown-Guedira, G., & Brown, J. (2020). Role of Effector-Sensistivity Gene Interactions and Durability of Quantitative Resistance to Septoria Nodorum Blotch in Eastern US Wheat. *Front. Plant Sci., 11*, 155.

Cresswell, H., & Kirkegaard, J. (1995). Subsoil ameriolation by plant roots -the process and evidence . *Australian Journal of Soil Research, 33*, 221-239.

Crute, I. R., & Pink, D. (1996). Genetics and utilization of pathogen resistance in plants. *Plant Cell, 8*, 1747–1755.

Czembo, P. e. (2003). QTL mapping of partial resistance in winter wheat to Stagnospora nodorum blotch. *Genome, 46*, 546-554.

Czembor, P., Arseniuk, E., Czaplicki, A., & al., e. (2003). QTL mapping of partial resistance in winter wheat to Stagnospora nodorum blotch . *Genome , 46*, 546-554.

DePauw, R. (1995). *Wheat improvement in Canada.* National Research Council/Plant Biotechnology Inst. Saskatoon, Canada: PBI Bulletin.

DePauw, R. (1995). *Wheat improvement in Canada.* National Research Council/Plant Biotechnology Inst. Saskatoon, Canada: PBI Bulletin.

Deveaux, Y., Toffano-Nioche, C., Claisse, G., & al, e. (2008). Genes of the most conserved WOX clade in plants affect root and flower development in Arabidopsis. *BMC Evol. Biol., 8*, 291.

Downie, R., & al., e. (2018). Assessing wheat sensitivities to Parastagnospora nodorum necrotrophic effectors and fine-mapping the Snn3-B1 locus conferring sensitivity to the effector SnTox3. *Front. Plant Sci., 9*, 881.

Dreisigacker, S., Sukumaran, S., Guzman, C., He, X., Bonnett, D., & Crossa, J. (2016). Molecular marker-based selection tools in spring wheat improvement: CIMMYT experience and prospects. *In Molecular breeding for sustainable crop improvement* (pp. 421-474). Chicago: Springer International Publishing.

Dubin, H. J. (1996). Breeding disease-resistant wheats for tropical highlands and lowlands. *Annu. Rev. Phytopathol. , 34*, 503 - 526.

Duveiller, E., Singh, R. P., & Nicol, J. M. (2007). The challenges of maintaining wheat productivity: pests, diseases, and potential epidemics. *Euphytica, 157*, 417-430.

Eagles, H. A., Cane, K., & Trevaskis, B. (2011). Veery wheats carry an allele of Vm-A1 that has implications for freezing tolerance in winter wheats . *Plant Breeding, 130*, 1-10.

Earl, D., & vonHoldt, B. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour., 4*, 359-361.

Ecker, R., Dinoor, A., & Cahaner, A. (1989). The inheritance of resistance to Septoria glume blotch. I. Common bread wheat, Triticum aestivum. *Plant Breed., 103*, 113-131.

Ellwood, S., Syme, R., Moffat, C., & Oliver, R. (2012). Evolution of the three Pyrenophora cereal pathogens: Recent divergence, speciation and evolution of non-coding DNA. *Fungal Genetics and Biology, 49*, 825-829.

Elshire, R., Glaubitz, J., Sun, Q., Poland, J., Kawamoto, K., Buckler, E., & Mitchell, S. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One, 6*, e19379.

Elshire, R., Glaubitz, J., Sun, Q., Polland, J., Kawamoto, K., Buckler, E., & Mitchell, S. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species . *PLoS One, 6*, e19379.

Endelman, J., & Jannink, J. (2012). Shrinkage estimation of the realized relationship matrix. *Genetics, 2*, 1405-1413.

Ergen, N. Z., Thimmapuran, J., Bohnert, H. J., & Budak, H. (2009). Transcriptomic pathways unique to dehydration tolerant relatives of modern wheat. *Funct. Integr. Genomics, 9*, 377-396.

Erson, E., Yu, J., & Buckler, E. (2007). Applications of linkage disequilibrium and association mapping in crop plants. *Genomics Assisted Crop Improvement*, 97-119.

Ersoz, E. S., Yu, J., & Buckler, E. S. (2007). *"Applications of linkage disequilibrium and association mapping in crop plants," in Genomics-assisted Crop Improvements.* (a. R. R. J. Varshney, Ed.) Berlin: Springer.

Ersoz, E., Yu, J., & Buckler, E. (2007). Applications of linkage disequilibrium and association mapping in crop plants. *Genomics assisted crop improvement*, 97-119.

Ersoz, E., Yu, J., & Buckler, E. (2007). Applications of linkage disequilibrium and association mapping in crop plants. *Genomics-assisted crop improvement*, 97-119.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Mol. Ecol., 14*, 2611-2620.

Eyal, Z. (1981b). Integrated control of Septoria diseases of wheat. *Plant Dis., 65*, 763-768.

Eyal, Z., & al., e. (1981b). Integrated control of Septoria diseases of wheat. *Plant Dis., 65*, 763-768.

Eyal, Z., Scharen, A., Prescott, J., & van Ginkel, M. (1981a). *The Septoria diseases of wheat: concepts and methods of disease management.* Texcoco: CIMMYT.

Eyal, Z., Scharen, A., Prescott, J., & van Ginkel, M. (1987). *The Septoria diseases of wheat: concepts and methods of disease management.* CIMMYT.

Eyal, Z., Scharen, A., Prescott, J., & van Ginkel, M. (1987a). *The Septoria diseases of wheat: concepts and methods of disease management.* Texcoco: CIMMYT.

FAO. (2013). *FAO statistical year book 2013: World food and agriculture.* Rome, Italy: FAO.

FAO. (2017). *Food and Agriculture Organization of the United Nations, Statistical Yearbook.* Rome : FAO.

FAOSTAT. (n.d.). *http://www.fao.org/faostat/en/data/QC*. Retrieved May 22, 2020

Faris, J., & Zhang, Z. e. (2010). A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. *Proceedings of the National Academy of Sciences USA, 107*, 13544-13549.

Faris, J., & Zhang, Z. e. (2010). A unique wheat disease resistance-like gene governs effector-triggered susceptibility to nectrophic pathogens. *Proceedings of the National Academy of Sciences USA, 107*, 13544-13549.

Faris, J., Zhang, Z., & al., e. (2010). A unique wheat resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. *Proceedings of the National Academy of Sciences USA, 107*, 13544-13549.

Figueroa, M., Hammond-Kosack, K., & Solomon, P. S. (2018). A review of wheat diseases-a field perspective. *Mol. Plant Pathol., 19*, 1523-1536.

Fischer, R. A., & Edmeades, G. O. (2010). Breeding and cereal yields progress . *Crop Sci., 50*, S85-98.

Flor, H. (1942). Inheritance for pathogenicity in Melampsora lini. *Phytopathol., 32*, 653-669.

Flor, H. (1955). Host-parasite interaction in flax rust, its genetics and other implications. *Phytopathology, 45*, 680-685.

Flor, H. (1956). The complementary genic systems in flax and flax rust. *Genet, 8*, 29-54.

Francki, M. (2013). Improving Stagonospora nodorum resistance in wheat: a review. *Crop Sci., 53*, 355-365.

Francki, M. (2013). Improving Stagonospora nodorum resistance in wheat: a review. *Crop Sci., 53*, 355-365.

Francki, M. G., Shankar, M., Walker, E., & al., e. (2011). New quantitative trait loci for flag leaf resistance to Stagnospora nodorum blotch. *Phytopathology, 101*, 1278-1284.

French, E., Kim, B., & Iyer-Pascuzzi, A. (2016). *Mechanisms of quantitative disease resistance in plants* (Vol. 56). New York: Academic Press.

French, E., Kim, B., & Iyer-Pascuzzi, A. (2016). Mechanisms of quantitative disease resistance in plants. *In Seminars in cell & development biology. 56*, pp. 201-208. Academic Press.

Friesen, T. L., & al., e. (2009). Host-selective toxins produced by Stagnospora nodorum confer disease susceptibility in adult wheat plants under field conditions. *Theor. Appl. Genet., 118*, 1489-1497.

Friesen, T. L., Chu, C. G., Liu, Z. H., & al., e. (2009). Host-selective toxins produced by Stagnospora nodorum confer disease susceptibility in adult wheat plants under field conditions . *Theor. Appl. Genet., 118*, 1489-1497.

Friesen, T., Chu, C., Xu, S., & Faris, J. (2012). SnTox5-Snn5: A novel Stagnospora nodorum effector-wheat gene interaction and its relationship with the SnToxA-Tsn1 and SnTox3-Snn3-B1 interactions. *Mol. Plant Pathol., 13*, 1101-1109.

Friesen, T., Holmes, D., Bowden, R., & Faris, J. (2018). ToxA is present in the US Bipolaris sorokiniana population and is a significant virulence factor on wheat harboring Tsn1. *Plant Dis., 102*, 2446-2452.

Friesen, T., Stukenbrock, E., & al., e. (2006). Emergence of a new disease as a result of interspecific virulence gene transfer. *Nature Genetics, 38*, 953-956.

Friesen, T., Stukenbrock, E., & al., e. (2006). Emergence of a new disease as a result of interspecific virulence gene transfer. *Nature Genetics, 38*, 953-956.

Friesen, T., Stukenbrock, E., Liu, Z., Meinhardt, S., Ling, H., Faris, J., . . . Oliver, R. (2006). Emergence of a new disease as a result of interspecific virulence gene transfer. *Nature Genetics, 38*, 953-956.

Friesen, T., Zhang, Z., Solomon, P., Oliver, R., & Faris, J. (2008). Characterization of the interaction of a novel Stagnospora nodorum host-selective toxin with a wheat susceptibility gene. *Plant Physiol., 146*, 682-693.

Friessen, T., & Faris, J. (2010). Characterization of the wheat-Stagnospora nodorum disease system: what is the molecular basis of this quantitative necrotrophic disease interaction? *Can. J. Plant Pathol., 32*, 20-28.

Fry, W. (1982). *Principles of plant disease management* . New York: Academic Press Inc.

Furbank, R. T., & Tester, M. (2011). Phenomics-technologies to relieve the phenotyping bottleneck. *Trends Plant Sci., 16*, 635-644.

Gahlaut, V., Jaiswal, V., Tyagi, B., & al, e. (2017). QTL mapping for nine drought-responsive agronomic traits in bread wheat under irrigated and rain-fed environments. *PLoS ONE, 12*, e0182857.

Gao, Y. e. (2015). Identification and characterization of the SnTox6-Snn6 interaction in the Parastagnospora nodorum-Wheat Pathosystem. *Mol. Plant-Microbe Interact., 28*, 615-625.

Gonzalez-Hernandez, J. L., Singh, P. K., Mergoum, M., & al., e. (2009). A quantitative trait loci on chromosome 5B controls resistance of Triticum turgidium (L.) var diccoides to Stagnospora nodorum blotch . *Euphytica, 166*, 199-206.

Gore, M., Wright, M., Ersoz, E., Bouffard, P., Szekeres, E., Jarvie, T., & Ware, D. (2009). Large-scale discovery of gene-enriched SNPs. *The Plant Genome, 2*, 121-133.

Gregory, P. J., Hutchson, D. J., Read, D. B., & al, e. (2003). Non-invasive imaging of roots with high resolution X-ray micro-tomography . *Plant Soil, 255*, 351-359.

Gregory, P. J., McGowan, M., Biscoe, P. V., & al., e. (1978). Water relations of winter wheat. 1: Growth of the root system. *J. of Agric. Sci., 91*, 91-102.

Grulke, N. (2011). The nexus of host and pathogens phenology: understanding the disease triangle with climate change. *New Phytologist, 189*, 8-11.

Gupta, P. K., Balyan, H. S., & Gahlaut, V. (2017). QTL analysis for drought tolerance in wheat: present status and future possibilities. *Agronomy, 7*, 5.

Gupta, P. K., Chand, R., Vasistha, N. K., Pandey, S. P., Kumar, U., Mishra, V. K., & al., e. (2018). Spot blotch disease of wheat: the current status of research on genetics and breeding. *Plant Pathol., 67*, 508–531.

Gupta, P. K., Langridge, P., & Mir, R. R. (2010). Marker-assisted wheat breeding: present status and future possibilities. *Mol. Breed. , 26*, 145-161.

Gupta, P. K., Rustgi, S., & Kulwal, P. L. (2005). Linkage disequilibrium and association studies in higher plants: present status and future prospects. *Plant Mol. Biol., 57*, 461-485.

Gupta, P., Kulwal, P., & Jaiswal, V. (2014). *Association mapping in crop plants: Opportunities and challenges: in Advances in Genetics* (Vol. 85). Academic Press Inc.

Gupta, P., Rustgi, S., & Kulwal, P. (2005). Linkage disequilibrium and association studies in higher plants: present status and future prospects. *Plant Molecular Biology, 57*, 461-485.

Gupta, P., Rustgi, S., & Kulwal, P. (2005). Linkage disequilibrium and association studies in higher plants: present status and future prospectus. *Plant Molecular Biology, 57*, 461-485.

Gurung, S., Mamidi, S., Bonman, J. M., & al., e. (2014). Genome-wide association study reveals novel quantitative trait loci associated with resistance to multiple leaf spot diseases of spring wheat. *PLoS One, 9*, e108179.

Gurung, S., Mamidi, S., Bonman, J., Xiong, M., Brown-Guedira, G., & al., e. (2014). Genome wide association study reveals novel quantitative trait loci associated

with resistance to multiple leaf spot diseases of spring wheat . *PLoS ONE, 9*, e108179.

Gurung, S., Mamidi, S., Bonman, J., Xiong, M., Brown-Guerdira, G., & Adhikari, T. (2014). Genome wide association study reveals novel quantitative trait loci associated with resistance to multiple leas spot diseases of spring wheat. *PLoSONE*, 9:e108179.

Guseman, J. M., Webb, K., Srinivavasan, C., & Dardick, G. (2017). DRO1 influences root system architecture in Arabidopsis and Prunus species. *Plant J., 89*, 1093-1105.

Guttieri, M., & al., e. (2015). Variation for grain mineral concentration in a diversity panel of current and historiacal Great Plains hard winter wheat germplasm. *Crop Sci., 2015*, 1035-1052.

Halder, J., & al., e. (2019). Mining and genomic characterization of resistance to tan spot, Stagnospora nodorum blotch (SNB) and Fusarium head blight in Watkins core collection of wheat landraces. *BMC Plant Biol., 19*, 1-15.

Hammer, G. L., Dong, Z., McLean, G., & al., e. (2009). Can changes in canopy and/or root system architecture explain historical maize yield trends in the US corn belt? *Crop Sci., 49*, 299-321.

He, Y., Mao, S., Gao, Y., & al, e. (2016). Genome-wide identification and expression analysis of WRKY transcription factors under multiple stresses in Brassica napus . *PLos ONE, 11*, e0157558.

Hendrick, R., & Pregitzer, K. (1992). Spatial variation in tree root distribution and growth associated with mini-rhizotrons . *Plant Soil, 143*, 283-288.

Henry, A., Gowda, V. R., Torres, R. O., & al., e. (2011). Variation of root system architecture and drought response in rice (Oryza sativa): phenotyping of the OryzaSNP panel in rainfed lowland fields. *Field Crops Res., 120*, 205-214.

Horbach, R., Navarro-Quesada, W., & Knogge, e. a. (2011). When and how to kill plant cell: infection strategies of plant pathogenic fungi. *J. Plant Physiol., 168*, 51-62.

Hund, A., Trachsel, S., & Stamp, P. (2009). Growth of axile and lateral roots of maize: I development of a phenotyping platform. *Plant Soil, 325*, 335-349.

Hurd, E. (1964). Root study of three wheat varieties and their resistance to drought and damage by soil cracking . *Canadian J. Plant Sci., 44*, 240-248.

Hyten, D., & Lee, D. (2016). *Plant genetic mapping techniques.* Chicago: eLS.

Iannucci, A., Marone, D., Russo, M. A., & al., e. (2017). Mapping QTL for root and shoot morphological traits in durum wheat x T. dicoccum segregating population at seedling stage. *Int. J. Genomics , 2017*, 17.

International Wheat Genome Sequencing Consortium (IWGSC); et al. (2018). Shifting the limits in wheat research and breeding using fully annotated reference genome. *Science*, eaar7191.

Iyer-Pascuzzi, A. S., Symonova, O., Mileyko, Y., & al, e. (2010). Imaging and analysis platform for automatic phenotyping and trait ranking of plant root systems. *Plant Physiol., 152*, 1148-1157.

Johal, G., & Briggs, S. (1992). *Reductase activity encoded by HM1 disease resistance gene in maize* (Vol. 258). Chicago.

Johnson, R. (1981). Durable resistance: definition of genetic control and attainment in plant breeding. *Phytopathology, 71*, 567-568.

Johnson, R., & Law, C. (1975). Genetic control of durable resistance to yellow rust (Puccinia striiformis) in wheat cultivar Hybrid de Bersee. *Annals of Appl. Biol., 81*, 385-391.

Jones, J., & Dangl, J. (2006). The plant immune system . *Nature, 444*, 323-329.

Joshi, A. K., Kumar, S., Chand, R., & Ortiz-Ferrara, G. (2004). Inheritance of resistance to spot blotch caused by Bipolaris sorokiniana in spring wheat. *Plant Breed., 123*, 213-219.

Kato-Noguchi, H. (2004). Allelopathic substance in rice root exudates: rediscovery of momilactone B as an allelochemical. *Journal of Plant Physiology, 161*, 271-276.

Keller, B., Wicker, T., & Krattinger, S. (2018). Advances in wheat pathogen genomics: implications for disease control. *Annu. Rev. Phytopathol., 56*, 67-87.

Khan, H., Tomar, S. M., & Chowdhury, S. (2010). Genetic analysis of resistance to spot blotch (Bipolaris sorokiniana) in wheat. *Indian J. Genet. Plant Breed., 70*, 11-16.

King, J., Cook, R., & Melville, S. (1983). A review of Septoria diseases of wheat and barley. *Ann. Appl. Biol., 103*, 345-373.

King, J., Cook, R., & Melville, S. (1983). A review of Septoria diseases of wheat and barley. *Ann. Appl. Biol. , 103*, 345 - 373.

Kirkegaad, J. A., Lilley, J. M., Howe, G. N., & al., e. (2007). Impact of subsoil water use on wheat yield. *Aust. J. Agric. Res., 58*, 303-15.

Kollers, S., Rodemann, B., Ling, J., & al., e. (2014). Genome-wide association mapping of tan spot resistance (Pyrenophora tritici-repentis) in European winter wheat. *Mol. Breed. , 34*, 363-371.

Korte, A., & Farlow, A. (2013). The advantages and limitations of trait analysis with GWAS: a review. *Plant Methods, 9*, 29.

Korte, A., & Farlow, A. (2013). The advantages and limitations of trait analysis with GWAS: a review. *Plant methods, 9*, 29.

Korte, A., & Farlow, A. (2013). The advantages and limitations of trait analysis with GWAS: a review. *Plant Methods, 9*, 29.

Krattinger, S., & Keller, B. (2016). Molecular genetics and eveolution of disease resistance in cereals. *New Physiologist, 212*, 320-332.

Krattinger, S., & Keller, B. (2016). Molecular genetics and evolution of disease resistance in cereals. *New Phytologist, 212*, 320-332.

Krupinsky, J., Tanaka, D., Merrill, S., Liebig, M., Lares, M., & Hanson, J. (2007). Crop sequence effects on leaf spot diseases of no-till spring wheat. *Agron. J., 99*, 912-920.

Kugler, K. (2013). Quantitative trait loci-dependent analysis of a gene co-expression network associated with fusarium head blight resistance in bread wheat (Triticum aestivum). *BMC Genomics, 14*, 1-30.

Kulkarni, M., Soolanayakanahally, R., Ogawa, S., & al, e. (2017). Drought response in wheat; key genes and regulatory mechanisms controlling root system architecture and transpiration efficiency. *Front. Chem., 5*, 106.

Kumar, S., Archak, S., Tyagi, R. K., & al, e. (2017). Evaluation of 19,460 wheat accessions conserved in the Indian national Gene bank to identify new sources of resistance to rust and spot blotch diseases. *PLoS One, 12*, e0175610.

Kumar, S., Tripathi, S. B., & Kumar, U. (2015). Dissection of wheat spot blotch disease resistance QTLs in to single Mendelian genes. *Indian J. Genet. Plant Breed., 75*, 434-439.

Kushwaha, U. K., Mangal, V., Bairwa, A. K., & al., e. (2017). Association mapping, principles and techniques. *J. Biol. Environ. Eng. , 2*, 1-9.

Lander, E., & Bostein, D. (1989). Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics , 121*, 185-199.

Leath, S., Scharen, A., Lund, R., & Dietz-Holmes, M. (1993). Factors associated with global occurences of Septoria nodurum blotch and Septora tritici blotch of wheat. *Plant Dis., 77*, 1266-1270.

Leath, S., Scharen, A., Lund, R., & Dietz-Holmes, M. (1993). Factors associated with global occurences of Septoria nodurum blotch and Septora tritici blotch of wheat. *Plant Dis. , 77*, 1266-1270.

Leng, Y. Q., Wang, R., Ali, S., & al., e. (2016). Sources and genetics of spot blotch resistance to a new pathotype of Cochliobolus sativus in the USDA National small grains collection. *Plant Dis., 100*, 1988–1993.

Li, B., Liu, D., Li, Q., & al, e. (2016). Overexpression of wheat gene TAMOR improves root system architecture and grain yield in Oryza sativa. *J. Exp. Bot., 67*, 4155-4167.

Liao, M., Palta, J. A., & Fillery, I. I. (2006). Root characteristics of vigorous wheat improve early nitrogen uptake. *Aust. J. Agric. Res., 57*, 1097-1107.

Lilley, J. M., & Kirkegaard, J. A. (2011). Benefits of increased soil exploration by wheat roots. *Field Crops Res., 122*, 118-30.

Lilley, J. M., & Kirkegaard, J. A. (2011). Benefits of increased soil exploration by wheat roots. *Field Crops Res., 122*, 118-130.

Liu, X., Huang, M., Fan, B., & al., e. (2016). Iterative usage of fixed and random effect models for powerful and efficient Genome-Wide Association Studies. *PLOS Genet., 12*, e1005767.

Liu, Z. e. (2006). The Tsn1-ToxA interaction in the wheat-Stagnospora nodorum pathosystem parallels that of the wheat-tan spot system. *Genome, 49*, 1265-1273.

Liu, Z. H., & al., e. (2004). Genetic and physical mapping of a gene conditioning sensitivity in wheat to a partially purified host-selective toxin produced by Stagnospora nodorum . *Phytopathology, 94*, 1056-1060.

Liu, Z., & al., e. (2015). Evaluation and Association Mapping of Resistance to Tan Spot and Stagnospora Nodorum Blotch (SNB) in adapted winter wheat germplasm . *Plant Dis., 99*, 1333-1341.

Liu, Z., Faris, J., & al., e. (2009). SnTox3 acts in effector triggered susceptibility to induce disease on wheat carrying the Snn3 gene. *PLoS Pathogens, e1000581*.

Liu, Z., Faris, J., & al., e. (2009). SnTox3 acts in effector triggered susceptibility to induce disease on wheat carrying the Snn3 gene. *PLoS, e1000581*.

Liu, Z., Faris, J., Meinhardt, S., & al., e. (2004). Genetic and physical mapping of a gene conditioning sensitivity in wheat to a partially puruWed host-selective toxin produced by Stagonospora ondorum. . *Phytophalogy, 94*, 1056 - 1060.

Liu, Z., Faris, J., Meinhardt, S., Ali, S., Rasmussen, J., & Friesen, T. (2004). Genetic and physical mapping of a gene conditioning sensitivity in wheat to a partially puruWed host-selective toxin produced by Stagonospora ondorum. *Phytopathology, 94*, 1056-1060.

Liu, Z., Friesen, T. L., Rasmussen, J. B., & al., e. (200b). Quantitative trait loci analysis and mapping of seedling resistance to Stagnospora nodorum leaf blotch in wheat . *Phytopathology, 94*, 1061-1067.

Liu, Z., Zhang, Z., Faris, J., Oliver, R., Syme, R., McDonald, M., . . . Friesen, T. (2012). The cystein rich necrotrophic effector SnTox1 produced by Stagonospora nodorum triggers susceptibility of wheat lines harbouring Snn1. *PLoS Pathol, 8*, e1002467.

Lobet, G., & Draye, X. (2013). Novel scanning procedure enabling the vectorization of entire rhizotron-grown root systems. *Plant Methods, 9*, 1.

Lopes, M. S., & Reynolds, M. P. (2010). Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. *Funct. Plant Biol., 37*, 147-56.

Lu, S., Gillian-Turgeon, B., & Edwards, M. (2015). A ToxA-like protein from Cochliobolus heterostrophus induces light-dependent leaf necrosis and acts as a virulence factor with host selectivity on maize. *Fungal Genet. Biol., 81*, 12-24.

Lucas, S. J., Salantur, A., Yazar, S., & Budak, H. (2017). High-throughput SNP genotyping of modern and wild emmer wheat for yield and root morphology using a combined association and linkage analysis . *Funct. Integr. Genomics , 17*, 667-685.

Ma, J., Li, R., Wang, H., & al, e. (2017). Transcriptomics analyses revealed wheat responses to drought stress during reproductive stages under field conditions . *Front. Plant Sci., 8*, 529.

Ma, J., Luo, W., Zhang, H., & al, e. (2017). Identification of quantitative trait loci for seedling root traits from Tibetan semi-wild wheat (Triticum aestivum ssp. tibetanum). *Genome, 25*, 1-8.

Mace, E. S., Singh, V., Van Oosterom, E. J., & al., e. (2012). QTL for nodal roott angle in sorghum (Sorghum bicolor L. Moench) co-locate with QTL for traits associated with drought adaptation. *Theor. Appl. Genet., 124*, 97-109.

Mairhofer, S., Zappala, S., Tracy, S., & al, e. (2013). Recovering complete plant root system architectures from soil via X-ray mu-computed tomography . *Plant methods, 9*, 8.

Manes, Y., Gomez, H., Puhl, L., & al., e. (2012). Genetic yield gains of the CIMMYT international semi-arid wheat yield trials from 1994 to 2010 . *Crop Sci., 52*, 1543-1552.

Manickavelu, A., Kawaura, K., Oishi, K., & al., e. (2012). Comprehensive functional analyses of expressed sequence tags in common wheat (Triticum aestivum) . *DNA Res., 19*, 165-177.

Manschadi, A. M., Christopher, J. T., Hammer, G. L., & al., e. (2010). Experimental and modelling studies of drought-adaptive root architectural traits in wheat (Triticum aestivum L.). *Plant Biosyst. Int. J. Deal Aspects Plant Biol, 144*, 458-62.

Manschadi, A., Hammer, G., Christopher, J., & al., e. (2008). Genotypic variation in seedling root architectural traits and implications for drought adaptation in wheat (Triticum aestivum L.). *Plant Soil, 303*, 115-29.

Manschadi, M. A., Christopher, J. T., DeVoil, P., & al., e. (2006). The role of root architectural traits in adaptation of wheat to water-limited environments. *Func. Plant Biol., 33*, 823-37.

Manske, G. G., & Vlek, P. L. (2002). *Root architecture- wheat as a model plant. Plant Roots; The Hidden Half* (Third ed.). (Y. Waisel, A. Eshel, & U. Kafkafi, Eds.) New York, USA: Marcel Dekker.

Mardis, A., & Elaine, R. (2008). The impact of next-generation sequencing technology on genetics. *Trends in genetics, 24*, 133-141.

Masi, C. E., & Maranville, J. W. (1998). Evaluation of sorghum root branching using fractals. *J. Agric. Sci. Cambridge, 131*, 259-265.

McCallum, M., Kirkegaard, J., Green, T., Cresswell, H., Davies, S., Angus, J., & Peoples, M. (2004). Improved subsoil macroporosity following perennial pastures . *Australian Journal of Experimental Agriculture, 44*, 299-307.

McDonald, M., Oliver, R., Friesen, T., Brunner, P., & McDonald, B. (2013). Global diversity and distribution of three necrotrophic effectors in Phaeosphaeria nodurum and related species. *New Phytologist, 199*, 241-251.

Mengiste, T. (2012). Plant immunity to necrotrophs. *Ann. Rev. Phytopathol., 50*, 267-294.

Merchuk-Ovnat, L., Fahima, T., Ephrath, J. E., & al, e. (2017). Ancestral QTL alleles from wild emmer wheat enhance root development under drought in modern wheat. *Front. Plant Sci. , 8*, 703-16.

Mergoum, M., & al., e. (2007). Reaction of elite wheat genotypes from the northern Great Plains of North America to Septoria diseases. *Plant Dis., 91*, 1310-1315.

Miedaner, T., & Korzun, V. (2012). Marker-assisted selection for disease resistance in wheat and barley breeding. *Phytopathology, 102*, 560-566.

Miyamoto, N., Steudle, E., Hirasawa, T., & Lafitte, R. (2001). Hydraulic conductivity of rice roots. *J. Exp. Bot., 52*, 1835-1846.

Mohan, M., Nair, S., Bhagwat, A., Krishna, T., Yano, M., Bhatia, C., & Sasaki, T. (1997). Genome mapping, molecular markers, and marker-assisted selection in crop plants. *Molecular Breeding, 3*, 87-103.

Mondal, B., Singh, A., Yadav, A., & al, e. (2017). QTL mapping for early ground cover in wheat (Triticum aestivum L.) under drought stress. *Curr. Sci. , 112*, 1266-1271.

Muehlchen, A. (1990). Evaluation of crucifer green manures for controlling Aphanomyces roor rot of peas. *Plant Diseases, 74*, 651-654.

Müller, T., Schierscher-Viret, B., Fossati, D., & al., e. (2018). Unlocking the diversity of genebanks: whole-genome marker analysis of Swiss bread wheat and spelt. *Theor. Appl. Genet. , 131*, 407-416.

Mundit, C. (2014). Durable resistance: a key to sustainable management of pathogens and pests. *Infect. Genet. Evol., 27*, 446-455.

Mwadzingeni, L., Shimelis, H., Dube, E., & al, e. (2016). Breeding wheat for drought tolerance: progress and technologies. *J. Integr. Agric., 15*, 61102-9.

Mwadzingeni, L., Shimelis, H., Rees, D. J., & al, e. (2017). Genome-wide association analysis of agronomic traits in wheat under drought-stressed and non-stressed conditions. *PLoS ONE, 12*, e0171692.

Nadia, K., Chang, X., & Jing, R. (2017). Genetic dissection of stem water-soluble carbohydrates and agronomic traits in wheat under different water regimes. *J. Agric. Sci., 9*, 42.

Nagel, K. A., Putz, A., Gilmer, F., & al, e. (2012). GROWSCREEN-Rhizo is a novel phenotyping robot enabling simultaneous measurements of root and shoot growth for plants grown in soil filled rhizotrons. *Funct. Plant Biol. , 39*, 891-904.

Nakamoto, T., & Oyanagi, A. (1994). The direction of growth of seminal roots of Triticum aestivum L. and experimental modification thereof. *Ann. Bot. , 73*, 363-7.

Nelson, L., & Gates, C. (1982). Genetics of host plant resistance of wheat to Septoria nodorum. *Crop Sci., 22*, 771-773.

Neumann, G., George, T., & Plassard, C. (2009). Strategies and methods for studying the rhizosphere- the plant science toobox. *Plant Soil, 321*, 431-456.

Ogura, T., & Busch, W. (2015). From phenotypes to causal sequences: using genome wide association studies to dissect the sequence basis for variation of plant development. *Curr. Opin. Plant Biol., 23*, 98-108.

Oliver, R. P., Tan, K. C., & Moffat, C. (2015). *Necrotrophic Pathogens of Wheat.* (Vols. 4-4). Elsevier Inc.

Oliver, R., Cai, X., & Wang, R. e. (2008). Resistance to tan spot and Stagnospora nodorum blotch in wheat-alien species derivatives . *Plant Dis., 92*, 150-157.

Oliver, R., Friesen, T., Faris, J., & Solomon, P. (2012). Stagonospora nodurum: from pathology to genomics and host resistance . *Annual Review of Phytopathology, 50*, 23-43.

Olofsdotter, M. (2001). Rice -a step toward use of allelopathy. *Agronomy Journal, 93*, 3-8.

Omori, F., & Mano, Y. (2007). QTL mapping of root angle in F2 populations from maize "B73" x teosonite "Zea luxurians". *Plant Root, 1*, 57-65.

Osman, M., He, X. Y., Benedettelli, S., & al., e. (2016). Identification of new sources of resistance to fungal leaf and head blight diseases of wheat. *Eur. J. Plant Pathol. , 145*, 305-320.

Ovenden, B., Milgate, A., Wade, L. J., & al, e. (2017). Genome-wide association for water-soluble carbohydrate concentration and relative maturity in wheat using SNP and DArT marker arrays. *Genes Genomes Genet., 7*, 2821-2830.

Oyanagi, A., & Nakamoto, T. (1993). Relationship between root growth angle of seedlings and vertical distribution of roots in the field in wheat cultivars. *Japanese J. Crop Sci., 62*, 565-570.

Palta, J. A., Chen, X., Milroy, S. P., & al., e. (2011). Large root systems: are they useful in adapting wheat to dry environments? *Funct. Plant Biol., 38*, 347-354.

Passioura, J. B. (1983). Rootd and drought resistance. *Agric. Water Management, 7*, 265-280.

Passioura, J. B. (2010). Scaling up: the essence of effective agricultural research. *Funct. Plant Biol., 37*, 585-591.

Pereira, D. A., McDonald, B., & Brunner, P. (2017). Mutations in the CYP51 gene reduce DMI sensitivity in Parastagnospora nodorum populations in Europe and China . *Pest Manag. Sci., 73*, 1503-1510.

Peremarti, A., Mare, C., Aprile, A., & al, e. (2014). Transcriptomic and proteomic analyses of a pale green durum wheat mutant shows variations in photosystem components and metabolic deficiencies under drought stress. *BMC Genomics, 15*, 125.

Peters-Haugrud, A., Zhang, Z., Richards, J., & al., e. (2019). Genetics of variable disease expression conferred by inverse gene-for-gene interactions in the wheat Parastagnospora nodurum pathosystem. *Plant Physiol., 180*, 420-434.

Phan, H., & al., e. (2018). Novel sources of resistance to Septoria nodorum blotch in the Vavilov wheat collection identified by genome-wide association studies. *Theor. Appl. Genet., 131*, 1223-1238.

Poland, J., & Rife, T. (2012). Genotyping-by-sequencing for plant breeding and genetics . *The Plant Geneome, 5*, 92-102.

Poorter, H., Buhler, J., van Dusschoten, D., & al, e. (2012). Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Funct. Plant Biol., 39*, 839-850.

Price, A. H., Townend, J., Jones, M. P., & al., e. (2002). Mapping QTLs associated with drought avoidance in upland rice grown in th ePhilippines and West Africa. *Plant Mol. Biol., 48*, 683-695.

Pritchard, J., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics, 155*, 945-959.

Qin, Y., Tian, Y., & Liu, X. (2015). A wheat salinity-induced WRKY transcription factor TaWRKY93 confers multiple abiotic stress tolerance in Arabidopsis thaliana . *Biochem. Biophys. Res. Commun., 464*, 428-433.

Quaedvlieg, W., Verkley, G., Shin, H., Barreto, R., Alfenas, A., Swart, W., . . . Crous, P. (2013). Sizing up Septoria. *Studies in mycology, 75*, 307-90.

Quaedvlieg, W., Verkley, G., Shin, H., Barreto, R., Alfenas, A., Swart, W., . . . Crous, P. (2013). Sizing up Septoria. *Studies in Mycology, 75*, 307-390.

Raboin, L.-M., Ballini, E., Tharreau, D., & al., e. (2016). Association mapping of resistance to rice blast in upland field conditions. *Rice, 9*, 59.

Ramakrishnan, S., Sidhu, J., & Ali, S. (n.d.). Molecular characterization of bacterial leaf streak resistance in hard red winter wheat. doi: 10.7717/peerj.7276.

Ramasamy, R., Ramasamy, S., Bindroo, B., & Naik, V. (2014). STRUCTURE PLOT: A program for drawing elegant STRUCTURE bar plots in user friendly interface. *Springerplus, 3*, 1-3.

Rasse, D., & Smucker, A. (1998). Root recolonization of previous root channels in corn and alfalfa rotations. *Plant and Soil, 204*, 203-212.

Ray, D., Gerber, J., MacDonald, G., & West, P. (2015). Climate variation explains a third global crop yield variability . *Nature communications, 6*.

Reddy, L., Friessen, T., Meinhardt, S., & al., e. (2008). Genomic analysis of the Snn locus on wheat chromosome arm 1BS and the identification of candidate genes. *Plant Genome, 1*, 55-66.

Richard, C. A., Hickey, L. T., Fletcher, S., & al, e. (2015). High-throughput phenotyping of seminal root traits in wheat. *Plant Methods, 11*, 13-24.

Richards, R. A., & Passiouura, J. B. (1989). A breeding program to reduce the diameter of the major xylem vessel in the seminal roots of wheat and its effect on grain yield in rain-fed environments . *Aust. J. Agric. Res., 40*, 943-950.

Richards, R. A., Watt, M., & Rebetzke, G. J. (2007). Physiological traits and cereal germplasm for sustainable agricultural systems . *Euphytica, 154*, 409-425.

Rimando, A., & Duke, S. (2003). *Studies on on rice allelochemicals. In: Rice: Origin, History, Technology and Production.* (C. Smith, Ed.) New York: John Wiley & Sons.

Rong, W., Qi, I., Wang, A., & al, e. (2014). The ERF transcriptional factor TaERF3 promotes tolerance to salt and drought stress in wheat. *Plant Biotechnol. J., 12*, 468-479.

Rooney, H., van't Klooster, J., van der Hoorn, R., Joosten, M., Jones, J., & de Wit, P. (2005). Cladosporium Avr2 inhibits tomato Rcr3 protease required for Cf-2-dependent disease resistance . *Science, 308*, 1783-1786.

Roth, C., Shroyer, J., & Paulsen, G. (2000). Allelopathy of sorghum on wheat under several tillage systems. *Agronomy Journal, 92*, 855-860.

Rowe, H., Renaut, S., & Guggisberg, A. (2011). RAD in the realm of next-gneration sequencing technologies . *Molecular Ecology, 20*, 3499-3502.

Rowse, H. R., & Goodman, D. (1981). Axial resistance to water movement in broad bean (Vicia faba) roots. *J. Exp. Botany, 32*, 591-598.

Roy, J. K., Smith, K. P., Muehlbauer, G. J., & al., e. (2010). Association mapping of spot blotch resistance in wild barley. *Mol. Breed., 26*, 243-256.

Ruud, A., & al., e. (2019). Genome-Wide Association Mapping of Resistance to Septoria nodorum leaf blotch in a Nordic Spring Wheat Collection . *Plant Genome, 12*, 180105.

Ruud, A., Windju, S., Belova, T., & al., e. (2017). Mapping of SnTox3-Snn3 as a major determinant of field susceptibility to Septoria nodorum leaf blotch in the SHA3/CBRD x Naxos population . *Theor. Appl. Genet., 130*, 1361-1374.

Saeed, I., Chen, X., Bachir, D. G., & al, e. (2017). Association mapping for photosynthesis and yield traits under two moisture conditions and their drought

indicies in winter bread (Triticum aestivum L.) using SSR markers. *Aust. J. Crop Sci., 11*, 248.

Sahu, B., Sumit, R., Srivastava, S., & Bhattacharyya, M. (2012). Sequence based polymorphic (SBP) marker technology for targeted genomic regions: its application in generating a molecular map of Arabidopsis thaliana genome. *BMC Genomics, 13*, 20.

Saintenac, C., & al., e. (2018). Wheat receptor-kinase-like protein Stb6 controls gene-for-gene resistance to fungal pathogen Zymoseptoria tritici. *Nat. Genet., 50*, 368-374.

Schnurbusch, T., Paillard, S., Fossati, D., & al., e. (2003). Detection of QTLs for Stagnospora glume blotch resistance in Swiss winter wheat. *Theor. Appl. Genet., 107*, 1226-1234.

Semagn, K., Bjostad, A., & Ndjiondjop, M. (2006). An overview of molecular marker methods for plants. *African Journal of Biotechnology, 5*, 25.

Shaner, G., & Buechley, G. (1995). Epidemiology of leaf spot of winter wheat caused by Septoria tritici and Stagonospora nodorum. *Plant Disease, 79*, 928-938.

Shaner, G., & Buechley, G. (1995). Epidemiology of leaf spot of winter wheat caused by Septoria tritici and Stagonospora nodorum. *Plant Dis., 79*, 928 - 938.

Shankar, M., Walker, E., Golzar, H., & al., e. (2008). Quantitative trait loci for seedling and adult plant resistance to Stagnospora nodorum in wheat . *Phytopathology, 98*, 886-893.

Sharma, R. C., Duveiller, E., Ortiz-Ferrara, G., & al., e. (2007b). Progress and challenge towards reducing wheat spot blotch threat in the Eastern Gangetic Plains of South Asia: Is climate change already taking its toll? *Field Crops Res., 103*, 109–118.

Sharma, R., & Duveiller, E. (2006). Spot blotch continuues to cause substantial grain yield reductions under resource-limited farming conditions. *J. Phytopathology, 154*, 482-488.

Sharp, R. E., Poroyko, V., Hejlek, L. G., & al., e. (2004). Root growth maintenance during water deficits: physiology to functional genomics. *J. Exper. Biol., 55*, 2343-2351.

Shi, G. e. (2015). The wheat Snn7 gene confers susceptibility or recognition of the Parastagnospora nodorum Necrotrophic Effector SnTox7. *Plant Genome , 8*, plantgenome2015.02.0007.

Shiferaw, B., Smale, M., Braun, H. J., & al., e. (2013). Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. *Food Secur., 5*, 291-317.

Sidhu, J. S., & al., e. (2020). Genome-Wide Association Study uncovers novel genomic regions associated with coleoptile length in hard winter wheat . *Front. Genet., 10*, 1345.

Solomon, P., Lowe, R., Tan, K.-C., Waters, O., & Oliver, R. (2006). Stagonospora nodurum: cause of stagonospora blotch of wheat. *Molecular Plant Pathology, 7*, 147-156.

Somers, D., Isaac, P., & Edwards, K. (2004). A high density microsatellite consensus map for bread wheat (Triticum aestivum L.). *Theoretical and Applied Genetics, 109*, 1105-1114.

Soriano, J. M., Malosetti, M., Rosello, M., & al, e. (2017). Dissecting the old Mediterranean durum wheat genetic architecture for phenology, biomass, and yield formation by association mapping and QTL meta-analysis. *PLoS ONE, 12*, e0178290.

Sukumaran, S., Dreisigacker, S., Lopes, M., & al., e. (2014). Genome-wide association study for grain yield and related traits in an elite spring wheat population grown in temperate irrigated environments. *Theor. Appl. Genet., 128*, 353-363.

Sun, D., Kertho, A., Mamidi, S., & al., e. (2015). Genome-wide association mapping for resistance to leaf and stripe rust in winter-habit hexaploid wheat landraces. *PLoS One 10:e0129580, 10*, e0129580.

Tan, K., Ferguson-Hunt, M., & al., e. (2012). Quantitative variation in effector activity of ToxA isoforms from Stagonospora nodurum and Pyrenophora tritici-repentis. *Molecular Plant-Microbe Interactions, 25*, 515-22.

Tan, K., Oliver, P., Solomon, P., & Moffat, C. (2010). Proteinaceous necrotrophic effectors in fungal virulence. *Functional Plant Biology, 37*, 907-912.

Tan, K., Oliver, R., Solomon, P., & Moffat, C. (2010). Proteinaceous necrotrophic effectors in fungal virulence. *Functional Plant Biology, 37*, 907-912.

Tang, Y., & al., e. (2016). GAPIT Version 2: An enhanced integrated tool for genomic association and prediction . *Plant Genome, 9*, plantgenome2015.11.0120.

Tennant, D., Scholz, G., Dixon, J., & Purdie, B. (1992). Physical and chemical characteristics of duplex soils and their distribution in the south-west of Western Australia. *Australian Journal of Experimental Agriculture, 32*, 827-843.

Thrall, P., & Burdon, J. (2002). Evolution of gene-gene systems in metapopulations: the effect of spatial scale of host and pathogen dispersal. *Plant Pathol., 51*, 169-184.

Tommasini, L., Schnurbusch, T., Fossati, D., Mascher, F., & al., e. (2007). Association mapping of Stagnospora nodorum blotch resistance in modern European winter wheat varieties. *Theor. Appl. Genet., 115*, 697-708.

Trachsel, S., Kaeppler, S., Brown, K., & al, e. (2011). Shovelomics: high-throughput phenotyping of maize (Zea mays L.) root architecture in teh field. *Plant Soil, 341*, 75-87.

Uga, Y., Okuno, K., & Yano, M. (2011). Dro 1, a major QTL involved in deeper rooting of rice under upland field conditions . *J. Exp. Bot., 62*, 2485-94.

Uga, Y., Sugimoto, K. O., & al., e. (2013). Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. *Nat. Genet. , 45*, 1097-102.

Uphaus, J., Walker, E., Shankar, M., & al., e. (2007). Quantitative trait loci identified for resistance to Stagnospora glume blotch in wheat in the USA and Australia . *Crop Sci., 47*, 1813-1822.

USDA-NASS. (2017). *United States Department of Agricultural Statistics Service, Report.* Retrieved from USDA-NASS: https://www.nass.usda.gov/Statistics_by_Subject

USDA-NASS. (2017). *USDA-NASS.* Retrieved from United States Department of Agriculture-National Agricultural Statistics Services, Report: https://www.nass.usda.gov/Statistics_by_Subject

Vamerali, T., Bandiera, M., & Mosca, G. (2012). *Minirhizotrons in modern root studies* . (S. Mancuso, Ed.) Berlin: Springer-Verlag.

Van Loon, L. (1997). Induced resistance in plants and the role of pathogenesis-related proteins. *European J. of Plant Pathol., 103*, 753-765.

Vasistha, N. K., Balasubramaniam, A., Mishra, V. K., & al., e. (2016). Enhancing spot blotch resistance in wheat by marker-aided backcross breeding. *Euphytica , 207*, 119-133.

Veyradier, M., Christopher, J., & Chenu, K. (2013). Quantifying potential yield benefit of root traits. In R. Sievanen, E. Nikinmaa, C. Godin, A. Lintunen, & P. Nygren (Ed.), *7th International Conference on Functional-Structural Plant Models*, (pp. 317-319). Saariselka, Finland.

Vleeshouwers, V., & Oliver, R. (2014). Effectors as tools in disease resistance breeding against biotrophic, hemibiotrophic and necrotrophic plant pathogens. *Mol. Plant-Microbe Interact., 27*, 196-206.

Wallace, J., & Mitchell, S. (2017). Genotyping-by-sequencing. *Current Protocols in Plant Biology*, 64-77.

Wang, C., Deng, P., Chen, L., & al, e. (2013). A wheat WRKY transcription factor TaWRKY10 confers tolerance to multiple abiotic stresses in transgenic tobacco. *PLoS ONE, 8*, e65120.

Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., & al., e. (2014). Characterization of polyploid wheat genomic diversity using a high density 90000 single nucleotide polymorphism array. *Plant Biotechnology J., 12*, 787-796.

Wang, X., Zeng, J., Li, Y., & al, e. (2015). Expression of TaWRKY44, a wheat WRKY gene, in transgenic tobacco confers multiple abiotic stress tolerance . *Front. Plant Sci., 6*, 615.

Wasson, A. P., Richards, R. A., Chatrath, R., & al, e. (2012). Traits and selection strategies to improve root systems and uptake in water-limited wheat crops. *J. Exp. Bot., 63*, 3485-3498.

Wasson, A. P., Richards, R. A., Chatrath, R., & al., e. (2012). Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. *J. Exp. Bot., 63*, 3485-98.

Waters, O., Lichtenzveig, J., Rybak, K., & al., e. (2011). Prevalence and importance of sensitivity to the Stagnospora nodorum necrotrophic effector SnTox3 in current Western Australian wheat cultivars. *Crop Pasture Sci., 62*, 556-562.

Wicki, W., Winzeler, M., Scmid, J., Stamp, P., & Messmer, M. (1999). Inheritance of resistance to leaf and glume blotch caused by Septoria nodorum Berk. in winter wheat. *Theor. Appl. Genet., 99*, 1265-1272.

Wilkinson, C., Murphy, J., & Rufty, R. (1990). Diallel analysis of components of partial resistance to Septoria nodorum in wheat. *Plant Dis., 74*, 47-50.

Winterberg, B., & al., e. (2014). The necrotrophic effector protein SnTox3 re-programs metabolism and elicites a strong defence response in susceptible wheat leaves. *BMC Plant Biol., 14*.

Xiao, Y., Liu, H., Wu, L., & al., e. (2017). Genome-wide association studies in maize: praise and stargaze. *Mol. Plant, 10*, 359-374.

Xu, S., Friessen, T., & Mujeeb-Kazi, A. (2004). Seedling resistance to tan spot and Stagnospora nodorum blotch in synthetic hexaploid wheats. *Crop Sci., 44*, 2238-2245.

Xu, Y. F., Li, S. S., Li, L. H., & al, e. (2017). QTL mapping for yield and photosynthetic related traits under different water regimes in wheat. *Mol. Breed., 37*, 34.

Xu, Z. S., Xia, L. Q., Chen, M., & al, e. (2007). Isolation and molecular characterization of the Triticum aestivum L. ethylene-responsive factor 1 (TaERF1) that increases multiple stress tolerance. *Plant Mol. Biol., 65*, 719-732.

Yu, J., & al., e. (2006). A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nat. Genet, 23*, 203-208.

Yu, J., Holland, J. B., McMullen, M. D., & al, e. (2008). Genetic design and statistical power of nested association mapping in maize. *Genetics, 178*, 539-551.

Zampieri, M., Ceglar, A., Dentener, F., & al., e. (2017). Wheat yield loss attributable to heat waves, drought and water excess at the global, national and subnational scales. *Enviorn. Res. Lett., 12*, 064008.

Zeng, Z. (1993). Theoretical basis for seperation of multiple linked gene effects in mapping quantitative trait loci. *Proceedings of the National Academy of Sciences , 90*, 10972-10976.

Zhang, Z., Friesen, T., Xu, S., Shi, G., Liu, Z., Rasmussen, J., & Faris, J. (2011). Two putatively homeoologous wheat genes mediate recognition of SnTox3 to confer effector-triggered susceptibility to Stagonospora nodurum . *Plant Journal, 65*, 27-38.

Zhao, Y., Hu, Y., Dai, M., & al, e. (2009). The WUSCHEL-related homeobox gene WOX11 is required to activate shoot-borne crown root development in rice. *The Plant Cell, 21*, 736-748.

CHAPTER -III

Genome-wide association analysis permits characterization of *Stagonospora nodorum* blotch (SNB) resistance in hard winter wheat

3.1 Introduction

Wheat (*Triticum aestivum L.*) is the largest grown cereal crop in the world and plays a crucial role in human food supply (FAOSTAT). Wheat demand is expected to surge by 60% to feed the projected population of 9 billion in 2050. However, wheat productivity is continuously constrained by biotic and abiotic factors including fungal diseases (Figueroa, Hammond-Kosack, & Solomon, 2018). Globally, these fungal diseases comprise wheat rusts, blights, and leaf spot diseases including Stagonospora nodorum blotch (SNB). SNB, caused by a necrotrophic fungus *Parastagonospora nodorum* (*Berk.*) {syn. *Septoria nodorum*, *Stagonospora nodorum*; teleomorph *Phaeosphaeria nodorum*) (Quaedvlieg, et al., Sizing up Septoria., 2013), is an important disease in most wheat-growing regions of the world (Oliver, Tan, & Moffat, 2015). The disease is quite common in Australia, the US and parts of northern Europe, causing significant yield losses (Oliver, Tan, & Moffat, 2015); (Francki, Improving Stagonospora nodorum resistance in wheat: a review, 2013); (Cowger, Ward, Brown-Guedira, & Brown, 2020); (Bhathal, Loughman, & Speijers, 2003). In the US, SNB is a recurrent disease of wheat in several geographic regions, including the Pacific Northwest, the upper seat Plains, and the Eastern states (Cowger, Ward, Brown-Guedira, & Brown, 2020). Adoption of no- or minimum tillage practices may further increase the incidence of disease in winter-wheat growing regions of the US. Fungicides are generally used to control SNB; however, there

have been several reports where high selection pressure among the pathogen populations has led to the development of resistance in the pathogen against several fungicides (Blixt, Djurie, Yuen, & Olson, 2009); (Pereira, McDonald, & Brunner, 2017). Thus, breeding for genetic resistance against SNB with reduced dependency on fungicides is a durable and environmental-friendly approach to manage SNB in wheat.

The biotrophic pathogens require living tissue and establish a long-term plant-pathogenic feeding relationship. To combat the biotrophic pathogens, plants have innate immune systems that activate the pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI) pathways (Jones & Dangl, 2006), which leads to the resistance following a classical gene-for-gene hypothesis (Flor H. , 1956). By contrast, *Parastagonospora nodorum* is a necrotrophic pathogen and its host interaction follows an inverse gene-for-gene model (Friesen, Stukenbrock, & al., 2006). In this case, the pathogen secretes proteins known as necrotrophic effectors (NEs) that interact with corresponding host sensitivity loci (*Snn*) and cause programmed cell death (Friesen, & al.,2007*)*. The first NE (*PtrToxA*) triggered susceptibility was observed in the wheat-*Pyrenophora tritici-repentis* pathosystem that causes tan spot in wheat germplasm carrying sensitivity gene *Tsn1* (Ballance, Lamari, & Bernier, 1989); (Faris, Zhang, & al., 2010). A nearly identical NE (*SnToxA*) was identified in *Parastagonospora nodorum* (Liu Z. e., 2006) with a corresponding host sensitivity gene, *Tsn1*. Compared to other NEs present in P. nodorum, *SnToxA* became an important virulence factor, which is believed to be horizontally transferred to *Pyrenophora tritici-repentis* (Friesen, Stukenbrock, & al., 2006) and also been identified in other fungi like *Cochliobolus*

heterostrophus (Lu, Gillian-Turgeon, & Edwards, 2015) and *Bipolaris sorokiniana* (Friesen, Holmes, Bowden, & Faris, 2018). In addition to *SnToxA*, there are several other NEs namely, *SnTox1, SnTox2, SnTox3, SnTox4, SnTox5, SnTox6, and SnTox7,* which interact with their corresponding *Snn* genes present in wheat *(Snn1, Snn2, Snn3, Snn4, Snn5, Snn6,* and *Snn7,* respectively) (Friesen, Stukenbrock, & al., 2006); (Abeysekera, Friesen, Keller, & Faris, 2009); (Friesen, Zhang, Solomon, Oliver, & Faris, 2008); (Friesen, Chu, Xu, & Faris, 2012); (Gao, 2015); (Shi, 2015); (Zhang, et al., 2011). Thus, SNB resistance in wheat largely depends on the presence of these susceptibility genes and is quantitatively inherited (Abeysekera, Friesen, Keller, & Faris, 2009).

Linkage analyses based on bi-parental populations has been useful to dissecting the genetic control of SNB resistance. This approach has identified several QTLs for SNB resistance on chromosomes (Friesen, Stukenbrock, & al., 2006); (Liu Z. e., 2006); (Abeysekera, Friesen, Keller, & Faris, 2009); (Gao, 2015); (Shi, 2015); (Arseniuk, 2004); (Czembo, 2003); (Friesen & al., 2009); (Liu & al., 2004); (Ruud, Windju, Belova, & al., 2017). These QTLs are a valuable resource for breeders to develop SNB resistant cultivars. However, However, linkage mapping can only encompass the allelic diversity segregating between the parents of the bi-parental population, limiting the scope of this approach (Gupta, Kulwal, & Jaiswal, 2014); (Korte & Farlow, 2013); (Ruud & al., 2019).

Genome-wide association studies (GWAS) or linkage disequilibrium-based mapping is another approach for dissecting the genetics of complex traits, which overcomes the major limitations of the linkage mapping. GWAS involves the evaluation of marker-trait associations (MTAs) in a large panel of unrelated individuals, harnessing the large

number of historical recombination (Korte & Farlow, 2013). GWAS have successfully uncovered several QTLs affecting yield, quality, biotic- and abiotic- stresses in wheat (Sidhu & al., 2020); (Ruud & al., 2019); (Ayana & al., 2018); (Halder & al., 2019); (Sukumaran, Dreisigacker, Lopes, & al., 2014). Several GWAS studies in wheat, which identified several QTLs for SNB resistance distributed over chromosomes 1A, 1B, 2A, 2D, 3A, 3B, 4A, 4B, 5A, 5B, 5D, 6A, 6B, 7A, and 7D (Ruud & al., 2019); (Halder & al., 2019); (Adhikari, Jackson, Gurung, Hansen, & al., 2011); (Gurung, Mamidi, Bonman, Xiong, Brown-Guedira, & al., 2014); (Liu & al., 2015); (Phan & al., 2018); (Tommasini, Schnurbusch, Fossati, Mascher, & al., 2007). These studies employed association-mapping panels comprising a large number of wheat landraces (Adhikari, Jackson, Gurung, Hansen, & al., 2011); (Gurung, Mamidi, Bonman, Xiong, Brown-Guedira, & al., 2014), a set of modern cultivars (Liu & al., 2015); (Tommasini, Schnurbusch, Fossati, Mascher, & al., 2007), and a historical set of wheat lines (Phan & al., 2018); however, most of these studies did not explore the US hard winter wheat cultivars/breeding materials.

In this study, we used a set of 274 accessions from the hard winter wheat association-mapping panel (HWWAMP) (Guttieri & al., 2015) to dissect the complexity of SNB resistance in hard winter wheat. The HWWAMP was successfully used in several GWA studies (Sidhu & al., 2020); (Ayana & al., 2018); (Guttieri & al., 2015); (Ramakrishnan, Sidhu, & Ali) to identify QTLs for disease resistance, grain quality traits, and coleoptile length. We screened the collection for resistance against SNB and sensitivity against *SnToxA*, *SnTox1*, and *SnTox3*. The objectives of the study were; (i) to identify and

evaluate the genetic basis of resistance against SNB; (ii) identify SNP markers associated with sensitivity to *SnToxA*, *SnTox1*, and *SnTox3*; (iii) identify candidate genes in the regions associated with SNB response.

3.2 Methods and materials

3.2.1 Plant materials

We used a set of 274 lines, selected from a hard winter wheat association mapping panel (HWWAMP) consisting of 299 accessions developed under the USDA Triticeae Coordinated Agricultural Project (TCAP) (Guttieri & al., 2015). The association mapping panel is composed of released varieties and breeding lines from the US Great Plains region including Colorado, Kansas, Michigan, Montana, Nebraska, North Dakota, Oklahoma, South Dakota, and Texas. Additional details about the HWWAMP accessions are available in the T3/Wheat database (https://triticeaetoolbox.org/wheat/pedigree/pedigree_info.php). Two differential lines, Salamouni (resistant to SNB) and Glenlea (susceptible to SNB), were included as checks for *Stagonospora nodorum blotch* (SNB) evaluations. The plant material used in this study did not require any permission/license for evaluation and all the necessary guidelines were followed.

3.2.2 Evaluations for seedling resistance to SNB

A set of 274 lines, along with two differential lines (Salamouni-SNB resistant and Glenlea-susceptible) were evaluated for Stagonospora nodorum blotch (SNB) reaction caused by *Parastagonospora nodorum* (isolate Sn2000) at the seedling stage under

greenhouse conditions in three independent experiments. The *Paratagonospora nodorum* isolate Sn2000 is reported to produce at least two host-selective toxins, SnTox1 and SnToxA (Liu, Z. *et al.,2006)*. In each of the experiments, all the lines were planted in a cone trainer (Ray Leach "Cone-trainer" ™ Single-Cell System) filled with Sunshine R 360 potting mixture (Sun Gro Horticulture, Agawam, MA, USA), with three plants per cone. The cones were placed in racks (Stuewe& Sons, Tangent, OR, USA) in a completely randomized design with three biological replicates.

A pure culture of isolate Sn2000 was grown on plates containing V8PDA medium and incubated at 21 °C under light for 7d. The plates were flushed with 30 mL sterile distilled water followed by scraping with a sterile glass slide to collect the pycnidiospores. The inoculum concentration was estimated using a hemocytometer and final concentration was adjusted to $1 \times 10^6\,mL^{-1}$ before inoculation.

Seedlings were spray inoculated at the two-leaf stage in the greenhouse using the method described by Abdullah et al (Abdullah & al., 2017) and placed in a humidity chamber with 100% humidity for 24 h to enhance the infection process. Thereafter, the plants were moved back to the greenhouse bench. Eight days after inoculation, the disease reactions were scored using a numerical scale of 0 to 5 based on the lesion type (Liu & al., 2004), where 0 = absence of visible lesions (highly resistant); 1 = few penetration points, with lesions consisting of flecking or small dark spots (resistant); and 5 = large coalescent lesions with very little green tissue remaining (highly susceptible).

3.2.3 Infiltrations with necrotrophic effectors (NEs)

All the 274 accessions along with the differential checks were grown as described above in three independent sets for infiltrations with toxins *SnToxA*, *SnTox1*, and *SnTox3*, respectively. Three fully expanded leaves of each accession were infiltrated with *SnToxA*, *SnTox1*, and *SnTox3* culture filtrates using a needle-less syringe following the methodology of (Faris et al 1996). Dr. Timothy Friesen, USDA-AS, Fargo, ND, kindly provided all the three NEs culture filtrates. Leaves of the seedlings were infiltrated with the equal volume (20–25 µl) of the filtrate. After 72h of infiltration, the seedlings were rated for infiltration responses. The sensitivity reactions were scored as sensitive = necrosis and tissue collapse; or insensitive = no reaction/necrosis.

3.2.4 Statistical analysis of phenotypic data

The linear mixed model (LMM) approach was used to analyze the phenotypic data for SNB inoculations, considering all factors as random. The data was analyzed based on the following model:

$$Y_{ijk}=\mu+G_i+E_j+GE_{ij}+R_{i(j)}+e_{ijk}$$

where "µ" stands for the population mean, "G" stands for genotypes, "E" for experiments, "R" for replications nested under experiments, and "e" for the random error. The analysis was performed in the R environment (Team.,2014). Correlation between effector sensitivity and SNB score was estimated in R. Different groups carrying the different number of resistant alleles were compared for allele stacking analysis using pairwise t-test with FDR (Benjamini,*et al*.,1995) correction in R.

3.2.5 SNP Genotyping

The HWWAMP was genotyped using the wheat Infinium 90K iSelect array (Illumina Inc. San Diego, CA) under the USDA-TCAP (Cavanagh & al., 2013) yielding a total of 21,555 SNPs. The genotypic data is publicly available and was obtained from the T3 Toolbox (https://triticeaetoolbox.org/wheat/genotyping/display_genotype.php?trial_code=TCAP90K_HWWAMP). As a quality control, the genotypic data were filtered with a minimum allele frequency (MAF) < 0.05 and more than 10% missing SNP data, leaving 15,590 SNP markers, which were used for further analysis. The genetic positions of the wheat Infinium 90K iSelect SNP markers were obtained from the consensus genetic map of 46,977 SNPs (Wang, Wong, Forrest, Allen, Chao, & al., 2014). The physical positions of the SNPs with significant associations with SNB were obtained by blasting the flanking sequences of respective SNPs to wheat Chinese Spring RefSeq v1.1 assembly (International Wheat Genome Sequencing Consortium (IWGSC); et al., 2018).

3.2.6 Population Structure and Linkage Disequilibrium

Population structure within the 274 HWWAMP accessions was inferred using a model-based Bayesian cluster analysis program, STRUCTURE v2.3.4 (Pritchard, Stephens, & Donnelly, 2000) to estimate the number of sub-populations. The admixture model was used with the number of assumed groups set from k = 1 to 10. The analysis was performed in five independent replicates, with 10,000 burn-in replicates and 10,000 Markov Chain Monte Carlo (MCMC) iterations in each of the runs. Structure Harvester (Earl & vonHoldt, 2012) was used to infer the optimum number of clusters using statistic

ΔK (delta K) (Evanno, Regnaut, & Goudet, 2005), which is based on the rate of change in the log probability of given data, between successive K values. The structure bar plot for the optimum number of clusters was drawn using Structure Plot v2.0 (Ramasamy, Ramasamy, Bindroo, & Naik, 2014). Linkage disequilibrium (LD) for the HWWAMP was analyzed using TASSEL v5.0 (Bradbury & al., 2007) with only 1,842 markers, taking out non-informative markers in our previous study (Ayana & al., 2018). The LD decay distances for the whole as well as individual genomes were estimated by plotting the r^2 values against the genetic distance (cM) between the markers.

3.2.7 Association mapping for SNB and NEs

Association analysis was performed using two different algorithms to select the model that better fits the data. The first was the MLM algorithm (with optimum compression and P3D), a single locus method (Yu & al., 2006), implemented in TASSEL (Trait Analysis by association, Evolution, and Linkage) v 5.0 software (Bradbury & al., 2007). The second model was Farm CPU (fixed and random model circulating probability unification) (Liu, Huang, Fan, & al., 2016), multilocus method implemented through Genomic Association and Prediction Integrated Tool (GAPIT) (Tang & al., 2016) in the R environment. Both of the two models took into account a K-PC model (Yu & al., 2006), by including the kinship and population structure as covariates to improve the statistical power of association analysis. Kinship (K) was estimated using the Centered IBS (identity by state) method (Endelman & Jannink, 2012). The first three Q-variates obtained through STRUCTURE analysis were used as covariates in the models.

Generally, MLM is used as it incorporates kinship and population structure as covariates

to minimize the confounding effects and controls the false positives. However, it leads to several false negatives due to the confounding between population structure and quantitative trait nucleotides (QTNs). We evaluated FarmCPU, an improved multiple-locus model (testing multiple markers simultaneously) that eliminates the drawbacks of the MLM algorithm by using associated markers as covariates to perform marker tests within a fixed-effect model. Further, it employs a separate random effect model to optimize the association between tested markers and the trait (Liu, Huang, Fan, & al., 2016).

These two algorithms were compared using the quantile-quantile (QQ) plots obtained from the analysis. The QQ plots suggested that Farm CPU performed better than MLM algorithm for Stagonospora nodorum blotch (isolate Sn2000) response data. Therefore, we used Farm CPU to detect the MTAs and identify candidate genes for SNB resistance using the grand mean of disease score from three independent experiments. The MLM algorithm fitted better on the effector infiltrations data. Thus, we used the best model to report the MTAs for each trait. The threshold for significance was corrected for multiple testing using a Bonferroni correction and False Discovery Rate (FDR) correction. Associations surpassing the corrected *p*-value were declared as significant MTAs.

3.2.8 Identification of candidate genes

The physical positions of all significant SNPs on Chinese spring (CS) RefSeq v1.1 were obtained from the IWGSC database by BLASTN searching the flanking sequences of

respective SNPs (International Wheat Genome Sequencing Consortium (IWGSC); et al., 2018). The gene models within +/-2 Mb of the most significant associated marker were derived from IWGSC RefSeq 1.1. The high confidence genes in the selected region were retrieved and IWGSC Functional Annotation v1.0 (International Wheat Genome Sequencing Consortium (IWGSC); et al., 2018). IWGSC Functional Annotation v1.0 (International Wheat Genome Sequencing Consortium (IWGSC); et al., 2018) was used to identify the genes with putative disease resistance functions based on a thorough review of the literature.

3.3 Results

3.3.1 The response of HWWAMP accessions to SNB

SNB resistance in 274 accessions of HWWAMP were evaluated for SNB resistance at the seedling stage in three independent experiments (Exp 1, Exp 2, and Exp3). These accessions exhibited a wide variation in response to SNB inoculations from highly resistant to fully susceptible genotypes (Fig 1A). The three experiments were statistically consistent based on the linear mixed model (LMM) analysis. Furthermore, a high correlation ($r > 0.90$) was observed among the three experiments. The overall mean and median disease scores for SNB infection were 2.95 and 3.00, respectively. The majority of the accessions (105 out of 274) had a disease score within a range of 3.0-3.9, indicating a moderately susceptible reaction, followed by 70 accessions with a disease score between 2.0-2.9 (moderately resistant). Moreover, 57 accessions showed a susceptible response with a disease score of 4.0-5.0, and 42 accessions have a disease score of less than 2, indicating a resistant response (Fig 1B).

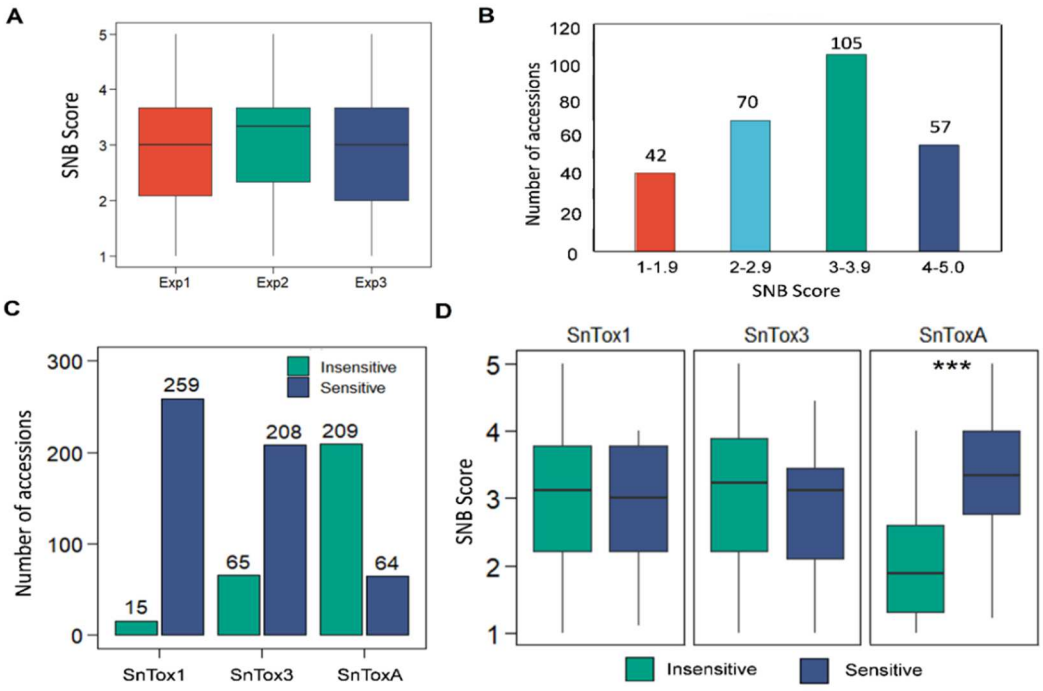

Figure 1. SNB response and necrotrophic-effectors sensitivity reaction of hard winter wheat association mapping panel (HWWAMP) accessions. **(A)** Boxplots showing the distribution of SNB scores of 274 HWWAMP accessions in the three experiments. **(B)** Disease distribution of Sn2000 inoculations in 274 accessions. **(C)** Sensitivity reaction of the 274 accessions against SnToxA, SnTox1, and SnTox3. **(D)** Boxplots for average SNB scores versus sensitivity reaction for three necrotrophic effectors. T-test was used for comparison between groups for SNB score. Asterisk denotes significant difference ($P < 0.01$)

Among the resistant lines, seven accessions were having a mean disease score of '1' across all three experiments (Table 1). Four of these seven lines ('Pioneer-2180', 'Colt', 'Sturdy-2-K', and 'TAM304') are varieties released after the 1980s from different breeding programs. The grand mean of SNB response for 274 HWWAMP accessions

recorded over three experiments is provided in Table2.

Table 1. Hard winter wheat association mapping panel (HWWAMP) accessions showing a high level of resistance against SNB, along with their mean disease score across three experiments

Accession	Year of Release	Origin	Pedigree	Disease Score	Number of 'R' alleles[1]
Pioneer-2180	1989	KS	TAM-101 / Pioneer W603 // Pioneer W558	1.0	5
COLT	1983	NE	Agate sib (NE69441)// (Tx65A1503-1) 391-56-D8 / Kaw	1.0	4
E2041	.	MI	Pioneer Brand 2552/Pioneer Brand 2737W	1.0	3
OK09634	.	OK	OK95616-98-6756/Overley	1.0	4
SD05210	.	SD	SD98444/SD97060	1.0	3
STURDY-2-K	2005	TX	Sinvalocho / Wichita // Hope / Cheyenne /3/2* Wichita /4/ Seu Seun 27	1.0	3
TAM304	2009	TX	TX92U3060/TX91D6564	1.0	4
NEKOTA	1994	NE	Bennett/TAM 107	1.1	3
OK05723W	.	OK	SWM866442/Betty	1.1	3

[1] The number of resistance-associated alleles at seven of the MTAs identified in this study.

Table 2. The sensitivity reaction against three Necrotrophic Effector (NE) for the accessions exhibiting a high level of resistance to SNB.

Accession	Necrotrophic Effector (NE) sensitivity			SNB score
	SnToxA	SnTox1	SnTox3	
Pioneer-2180	Insensitive	Insensitive	Insensitive	1.0
STURDY-2-K	Insensitive	Insensitive	Insensitive	1.0
Colt	Insensitive	Insensitive	Sensitive	1.0
TAM304	Insensitive	Insensitive	Insensitive	1.0
E2041	Insensitive	Insensitive	Insensitive	1.0
OK09634	Insensitive	Insensitive	Insensitive	1.0
SD05210	Insensitive	Insensitive	Insensitive	1.0
NEKOTA	Insensitive	Insensitive	Insensitive	1.1
Shocker	Insensitive	Insensitive	Insensitive	1.4
Darrel	Insensitive	Insensitive	Insensitive	1.5

3.3.2 Effector sensitivity and SNB reactions

In addition to SNB infection, we evaluated all 274 accessions for necrotrophic effector sensitivity. Three effector toxins namely *SnToxA*, *SnTox1*, and *SnTox3* were used to infiltrate all the accessions, independently. For *SnToxA*, 209 accessions were sensitive and 64 were insensitive. For *SnTox3*, there were 65 sensitive and 208 insensitive accessions. We did not find sufficient variation in the case of *SnTox1* as 259 accessions were insensitive and only 15 accessions were sensitive (Figure 1C). Furthermore, we

determined whether the effector sensitivity contributes to the SNB infection by comparing the infection and infiltration data. We found a significant difference for SNB disease severity among SnToxA sensitive and insensitive groups ($P < 2.2e-16$) at $P < 0.01$ level of significance (Figure 1D). The mean SNB score were 1.99 and 3.26 for insensitive and sensitive groups, respectively, indicating that *SnToxA*-sensitive accessions were significantly more susceptible than *SnToxA*-insensitive accessions. Contrary to *SnToxA*, we did not find any significant differences among sensitive and insensitive groups for SnTox1. The mean SNB score was 2.95 and 2.90 for insensitive and sensitive groups, respectively. As the isolate Sn2000 lacks SnTox3, the mean SNB score was similar for insensitive and sensitive groups (3.00 and 2.86) as expected (Fig. 1D).

3.3.3 Population structure and LD analysis

Before performing GWAS, we inferred the population structure among the 274 accessions based on model-based Bayesian clustering in STRUCTURE using 15,590 SNP markers. Population structure analysis revealed the existence of three subpopulations (P1, P2, and P3 for later reference) within the 274 accessions based on Delta K statistic. The three subpopulations i.e., P1, P2, and P3 consisted of 81, 152, and 41 accessions, respectively (Fig. 2). We attempted to determine the relationship between these subpopulations and the breeding program from which these accessions originated. Most of the accessions originating from South Dakota and Nebraska and all the accessions from Montana belonged to subpopulation P2. The accessions from the Colorado breeding program dominated subpopulations P1 and P2, with no accession in P3. Contrary to this, the accessions from Kansas, Oklahoma, and Texas were evenly

distributed among all three subgroups. The mean SNB score for P1, P2, and P3 was 3.05, 2.98, and 2.60, respectively, indicating that accessions from P1 and P2 incline towards moderately susceptible reaction and P3 being moderately resistant.

Linkage Disequilibrium (LD) analysis for HWWAMP accessions has already been performed using the same set of SNP markers in our previous study. We estimated the LD decay based on the r^2 values for the whole genome and individual genomes. The distance where LD value (r^2) decreases below 0.1 or half strength of D' (D' = 0.5) was estimated based on the curve of the nonlinear logarithmic trend line. LD decay was estimated to be 4.5 cM for whole-genome, whereas LD decay was around 3.4, 3.6 cM, and 14.2 cM in A and B and D genomes, respectively.

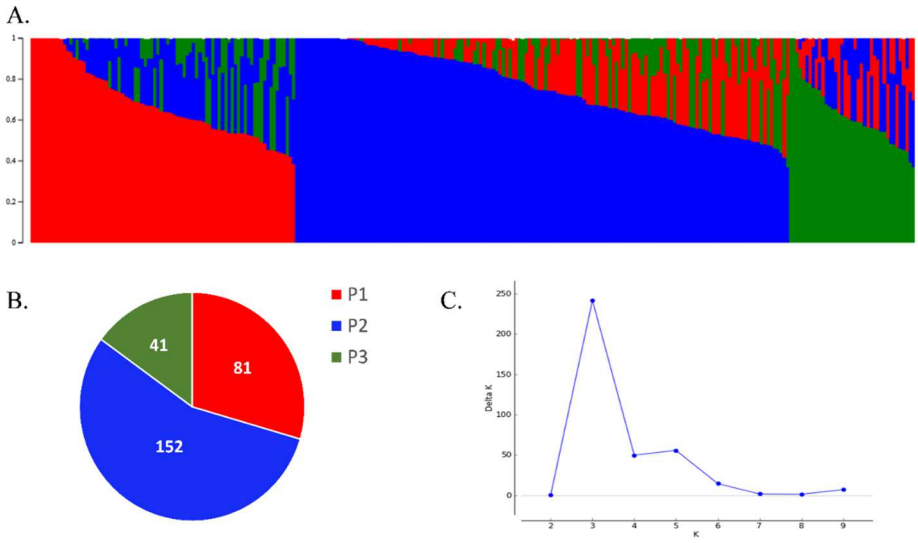

Figure 2. Structure analysis for the 274 accessions of hard winter wheat association mapping panel (HWWAMP). **(A)** Structure plot showing three subpopulations at K = 3. **(B)** Pie chart representing the number of accessions in each of the subpopulations. **(C)** the Delta K estimate for differing number of subpopulations (k).

3.3.4 GWAS for SNB and necrotrophic effectors (NEs)

Association analysis was performed in hard winter wheat panel for SNB and effector (NE) sensitivity, and MTAs were identified for respective phenotypes. Two different algorithms, namely MLM and FarmCPU were initially compared to select the best algorithm for further association analysis. The best algorithm was selected for each trait by comparing the model fitness by analyzing the QQ plots (Fig. 3). FarmCPU better fit the SNB response, while MLM was selected for the SnToxA, SnTox1, and SnTox3 infiltrations. The best model was used to report significant MTAs for each trait based on a genome-wide significance threshold of $P < 2.34 \times 10^{-6}$ ($-\log_{10} P > 5.50$) after Bonferroni correction of *P*-values, which is highly conservative and reduces the type I errors.

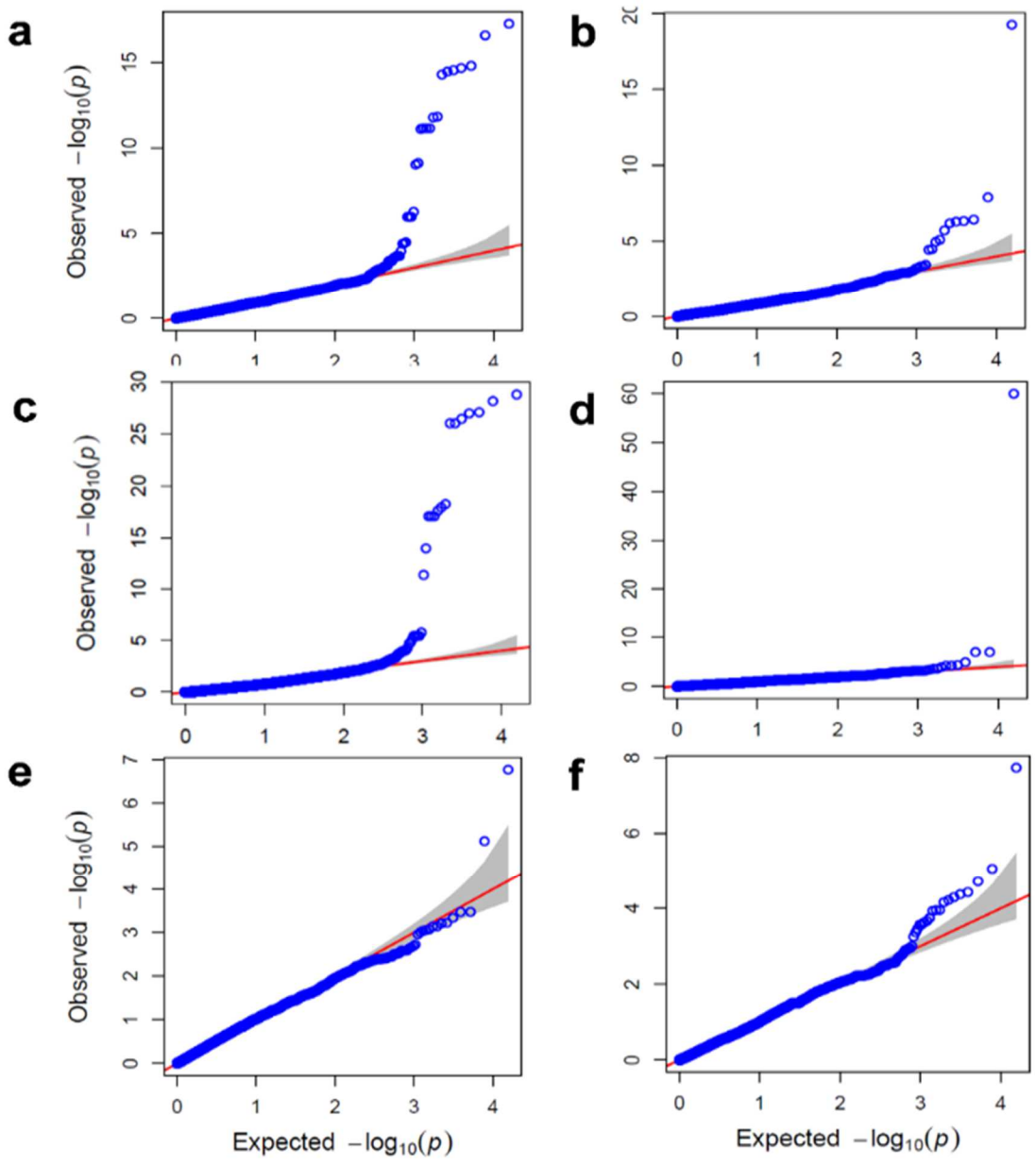

Figure 3. Quantile-quantile (QQ) plots of association analysis for different traits. (a) MLM model fitted on SNB infection data; (b) FarmCPU model fitted on SNB infection data; (c) MLM model fitted on SnToxA infiltration data; (d) FarmCPU model fitted on SnToxA infiltration data; (e) MLM model fitted on *SnTox3* infiltration data; and (f) FarmCPU model fitted on *SnTox3* infiltration data

GWAS for SNB response identified a total of seven significant MTAs for SNB resistance/susceptibility (Table 3; Fig. 4). The seven MTAs, representing seven distinct QTLs, were distributed on chromosomes 1B, 2AL, 2DS, 4AL, 5BL, 6BS, and 7AL (Table 3). The QTL (*QSnb.sdsu-5B*) with the largest effect was detected on chromosome 5BL, which corresponds to the genomic location of the susceptibility locus *Tsn1*. The most significant SNP (*tplb0027f13_1346*) for this association was physically mapped to 546 Mb on chromosome 5B (IWGSC RefSeq v1.1), which co-localized with the location of *Tsn1*. The second most significant association was detected near the distal end of the long arm of chromosome 7A, which seems to be robust QTL (*QSnb.sdsu-7A*) imparting SNB resistance/susceptibility (Table 3). The most significant SNP (*Excalibur_c6101_608*) for this association showed high significance ($-\log_{10}P = 7.89$) and a marker effect of 0.39. Apart from these, five more associations were declared significant after Bonferroni corrected *p*-values. Another MTA identified on the chromosome 2DS, which mapped at 9Mb on the physical map (IWGSC RefSeq v1.1), co-localizes with the known locus *Snn2* (6-12 Mb). All the significant associations are enlisted in Table 3, along with their physical location and corresponding marker effect.

Table 3. Summary of the significant markers associated with SNB resistance and necrotrophic-effector sensitivity. All the markers were declared significant based on Bonferroni corrected significance threshold -log10 P > 5.50.

Trait	SNP[a]	Allele	Chromosome	Position[b]	effect	*P*-value	FDR Adj (*P*-value)	-Log$_{10}$(*P*)
SNB	*IWA3048*	G/A	1B	364419320	0.2252	1.94e-06	0.0043	5.7124
SNB	*BS00024643_51*	C/T	2AL	779207329	-0.1953	3.91e-07	0.0016	6.4080
SNB	*D_contig17313_245*	C/A	2DS	9343858	0.2222	5.13e-07	0.0016	6.2897
SNB	*Kukri_rep_c107387_161*	A/G	4AL	742034488	-0.2928	6.59e-07	0.0017	6.1811
SNB	*tplb0027f13_1346*	C/T	5BL	546827934	-0.4680	5.60e-20	8.74e-16	19.2514
SNB	*RAC875_c55270_272*	A/G	6BS	30036111	0.1908	4.61e-07	0.0016	6.3362
SNB	*Excalibur_c6101_608*	T/C	7AL	721174878	0.3999	1.31e-08	0.0001	7.8819
SnToxA	*IACX9261*	T/G	5BL	546704036	0.4079	1.41e-29	2.19e-25	28.8500
SnTox3	*BS00032003_51*	G/A	5BS	2559360	-0.1674	1.72e-07	0.0026	6.7700

[a] SNP markers are from Infinium 90K array-based SNPs (Wang et al., 2015)

[b] Physical location is based on IWGSC RefSeq v 1.1 (2018)

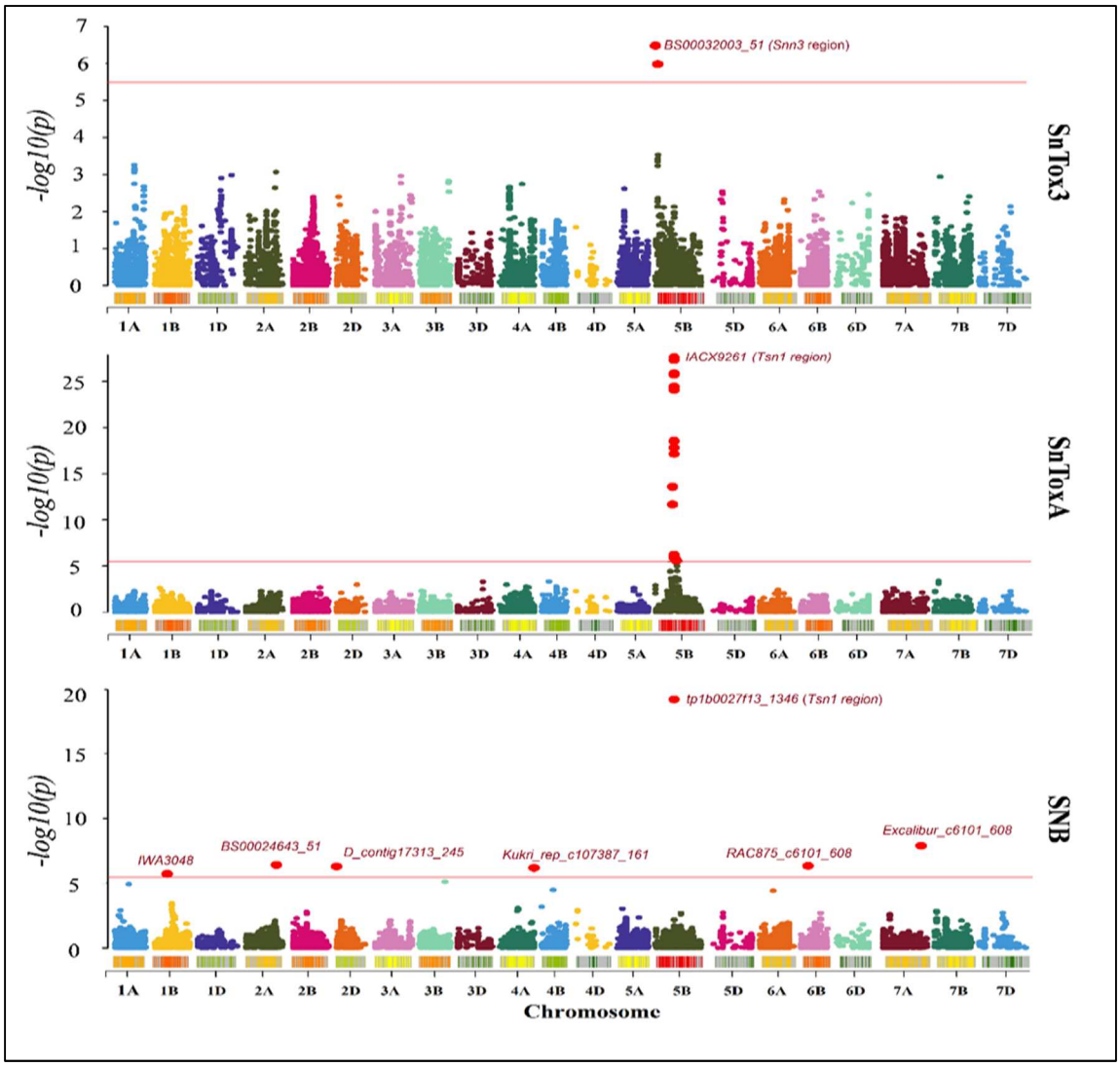

Figure 4. A Manhattan plot representing the marker-trait associations (MTAs) identified for SNB response and NEs reactions. **(A)** The MTAs for SnTox3 infiltrations, **(B)** MTAs for SnToxA infiltrations; **(C)** and MTAs for SNB response. The color scale indicates SNP density on the bar given in the inset. The red line depicts the Bonferroni corrected threshold for identifying significant associations. The significant associations are represented with red dots.

In addition to SNB response, MTAs were detected for SnToxA and SnTox3 infiltrations based on Bonferroni corrected genome-wide significance threshold. A single genomic region was identified on the long arm of chromosome 5B for SnToxA, which again co-locates with the genomic region of Tsn1. The physical location of the most significant SNP for SnToxA is the same as that detected on chromosome 5BL for SNB inoculations (Table 3). Furthermore, we identified a significant association for SnTox3 sensitivity on the short arm of chromosome 5B. The comparison of the physical locations of MTAs detected for SNB response and SnToxA on chromosome 5B with the MTA for SnTox3 showed that these associations are present on different 5B arms (Fig. 5). The association for SnTox3 mapped around the 3Mb region on the chromosome 5B in the physical map, whereas the SnToxA has mapped around 546 Mb (IWGSC RefSeq v1.1). The SnTox3 associated region in our study corresponded to the reported physical location of the Snn3 gene, corroborating several other reports for association in this region (Table 3; Fig 5). Contrary to SnToxA and SnTox3, no significant association was detected in the case of SnTox1 infiltrations, owing to the low variation for SnTox1 sensitivity among HWWAMP accessions.

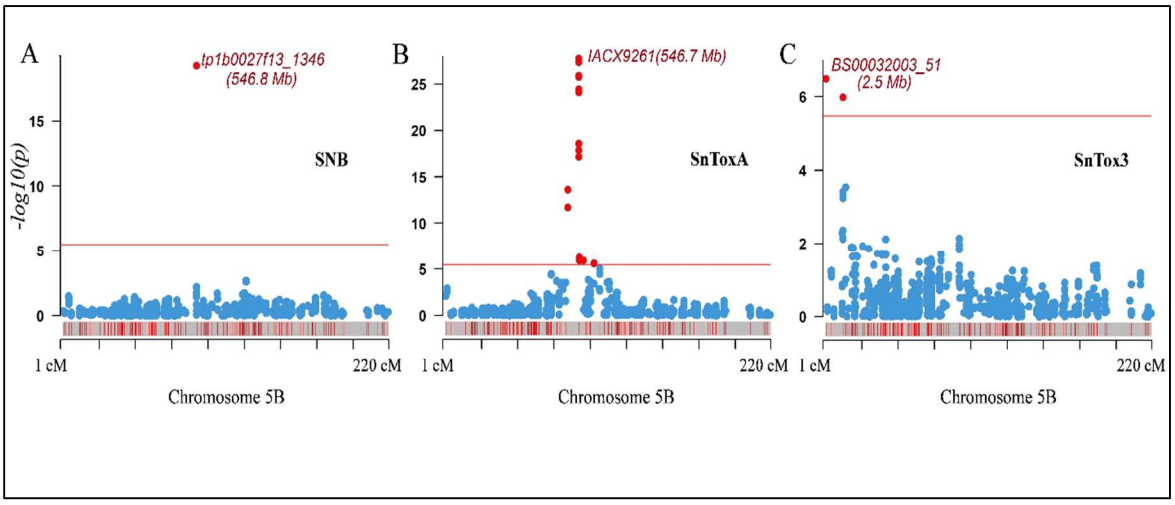

Figure 5. Marker-trait associations detected on chromosome 5B for **(A)** SNB inoculations, **(B)** SnToxA infiltrations, and **(C)** SnTox3 infiltrations. The color scale indicates SNP density on the bar given in the inset. The red line depicts the Bonferroni corrected threshold to declare significant associations. The physical location for the most significant SNP has been provided based on IWGSC RefSeq v 1.1 (2018).

3.3.5 Allele stacking analysis

The nature of SNB resistance is complex and governed by several active NE-receptor interactions which could vary in different environments. Thus, we studied the effect of accumulation of resistant alleles at seven of the detected QTLs, including *Tsn1* and *Snn2*. Different accessions from the HWWAMP were grouped based on the number of resistant alleles they carry for these seven QTLs. Although seven MTAs were detected in GWAS for SNB response, only six groups of accessions were identified in total, carrying zero to five resistance alleles (Fig. 6).

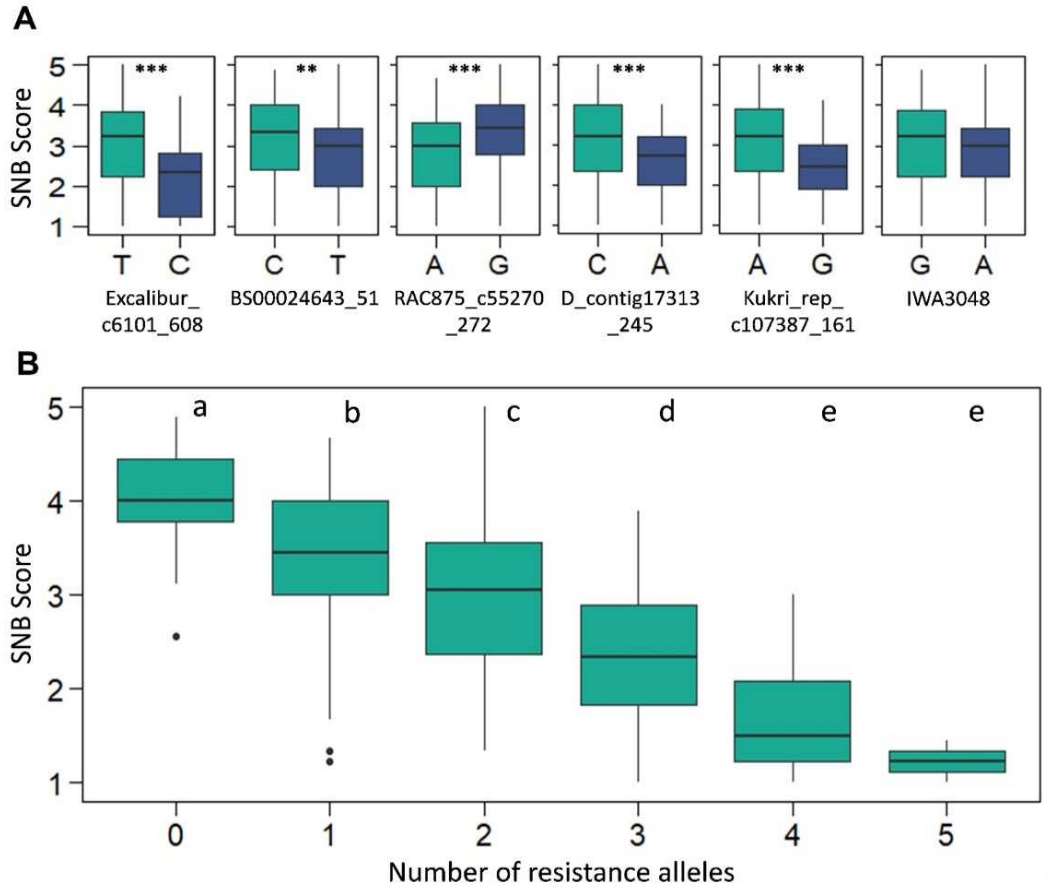

Figure 6. (**A)** Pairwise comparison for SNB score among two alleles of the seven significant MTAs identified for SNB resistance on chromosomes 5BL, 7AL, 2AL, 6BS, 2DS, 4AL, and 1B (enlisted in Table 3). A t-test was used to compare the two groups. Two asterisks denote significant difference at ($P < 0.01$) and three asterisks denote significant difference at ($P < 0.001$). **(B)** Effect of accumulation of resistant alleles for the detected associations on SNB disease score. The different groups were compared using FDR corrected pairwise t-test. Levels denoted by different letters are significantly different ($P < 0.05$)

None of the accessions of HWWAMP carry all seven favorable alleles. The mean and median SNB scores for the accessions (group 0) carrying no resistance allele were 4.11 and 4.00, respectively. On the other hand, group 5 comprising two accessions and having resistant alleles at five of the seven loci showed a mean and median SNB score of 1.22 and 1.20, respectively. Similarly, the group of accessions (group 4) with four resistant lines had a mean and median SNB score of 1.66 and 1.50, respectively. Furthermore, these groups (group 0 - group 5) were compared using FDR corrected pairwise t-test to verify the additive effect of the resistant alleles on SNB reaction. The differences in mean SNB scores were statistically significant and the accessions that carried a higher number of resistant alleles were having the lower mean SNB scores and vice versa.

3.3.6 Exploring the candidate genes for SNB resistance

We used five out of the seven MTAs (except the 5BL region corresponding to *Tsn1* and a potential association on chromosome 1B) identified in GWAS for SNB to explore the putative candidate genes. For each MTA, a two Mb window was used to identify the candidate genes. In total, we identified 166 High Confidence genes for the five MTAs based on CS RefSeq 1.1. The functional annotation for these genes was retrieved from IWGSC RefSeq 1.0 annotation. This led to the identification of 35 high confidence genes predicted to have a plant-disease related function based on a thorough review of the literature (Table 4). In the region spanning QTL *QSnb.sdsu-7A*, we identified three protein-kinase and one receptor kinase domain encoding genes. Similarly, four genes were found in *QSnb.sdsu-2A* region, including two NBS-LRR family proteins encoding

genes, that could be used to find the genes for this QTL. The *QSnb.sdsu-6B* region harbored five genes of importance, including an NBS-LRR protein and a receptor-like kinase. The region spanning the fourth QTL, *QSnb.sdsu-2D*, consisted of 14 genes with 12 NBS-LRR domain encoding genes and two genes with a protein-kinase domain. Furthermore, eight putative candidate genes with predicted role in plant defense response were identified in the region covering *QSnb.sdsu-4A*. (Table 4).

Table 4. Summary of the candidate genes in the identified QTL regions. Gene IDs and functional annotation are based on IWGSC CS RefSeq v 1.1 (2018)

QTL	Chromosome	Gene	Functional Annotation
QSnb.sdsu-2A	2A	TraesCS2A02G589900	Receptor-like kinase
	2A	TraesCS2A02G590100	Disease resistance protein: NB-ARC
	2A	TraesCS2A02G590200	Disease resistance protein RPM1: NB-ARC
	2A	TraesCS2A02G593500	Receptor kinase, putative
	2D	TraesCS2D02G018300	NB-ARC domain-containing disease resistance protein
QSnb.sdsu-2D	2D	TraesCS2D02G018400	NB-ARC domain-containing disease resistance protein
	2D	TraesCS2D02G018500	NB-ARC domain-containing disease resistance protein
	2D	TraesCS2D02G019200	NB-ARC domain-containing disease resistance protein
	2D	TraesCS2D02G019400	NB-ARC domain-containing disease resistance protein
	2D	TraesCS2D02G019500	NB-ARC domain-containing disease resistance protein
	2D	TraesCS2D02G019700	NB-ARC domain-containing disease resistance protein
	2D	TraesCS2D02G019800	Receptor-like protein kinase
	2D	TraesCS2D02G019900	NB-ARC domain-containing disease resistance protein
	2D	TraesCS2D02G020000	NB-ARC domain-containing disease resistance protein
	2D	TraesCS2D02G020300	NB-ARC domain-containing disease resistance protein
	2D	TraesCS2D02G020400	NB-ARC domain-containing disease resistance protein
	2D	TraesCS2D02G020700	NBS-LRR-like resistance protein
	2D	TraesCS2D02G021400	Receptor-like protein kinase
QSnb.sdsu-4A	4A	TraesCS4A02G496700	NBS-LRR-like resistance protein
	4A	TraesCS4A02G496800	NBS-LRR-like resistance protein
	4A	TraesCS4A02G497100	disease resistance protein (TIR-NBS-LRR class) family

	4A	TraesCS4A02G497300	Receptor-like protein kinase
	4A	TraesCS4A02G497800	receptor kinase 1
	4A	TraesCS4A02G497900	Receptor-like kinase
	4A	TraesCS4A02G499000	Receptor-like kinase
	4A	TraesCS4A02G499400	Receptor-like kinase
QSnb.sdsu-6B	6B	TraesCS6B02G050300	NBS-LRR resistance-like protein
	6B	TraesCS6B02G050800	Protein kinase family protein
	6B	TraesCS6B02G050900	Protein kinase family protein
	6B	TraesCS6B02G051000	Protein kinase family protein
	6B	TraesCS6B02G051700	Receptor-kinase, putative
QSnb.sdsu-7A	7A	TraesCS7A02G544000	Protein kinase family protein
	7A	TraesCS7A02G544100	Serine/threonine-protein kinase
	7A	TraesCS7A02G544200	Serine/threonine-protein kinase
	7A	TraesCS7A02G544600	receptor kinase 1

3.4 Discussion

SNB is an important fungal disease of wheat and a severe infection can cause significant yield losses. Thus, exploring the resistance sources among the existing germplasm and utilizing them in wheat breeding could be an effective disease management strategy. In this study, we used a US hard winter wheat association-mapping panel (HWWAMP), which turned out to be a good source of SNB resistant germplasm.

A total of 274 accessions of HWWAMP were used to evaluate for resistance against SNB. Out of 274 lines, 112 (40.87%) lines were identified as resistant or moderate-resistant to SNB, indicating novel or existing sources of resistance present in the HWWAMP. Around 50% of tested germplasm, including spring wheat and winter wheat, was found resistant to SNB in the previous studies (Halder & al., 2019) ; (Oliver, Cai, &

Wang, 2008) . Lines with SNB resistance were present among all breeding programs from Colorado, Kansas, Montana, Nebraska, Oklahoma, South Dakota, and Texas (Fig. 7). Interestingly, most of the breeding programs from where the accessions were collected have developed a few highly resistant germplasms against SNB. However, it is important to note that about 60% of the tested germplasm were susceptible to SNB in this study. One possible explanation for wide susceptibility in hard winter wheat breeding programs of the central U.S. states is retention of Tsn1, which could be a result of deliberate selection of some other resistance gene and/or likely linkage to important agronomic traits such as hardiness (Cowger, Ward, Brown-Guedira, & Brown, 2020).

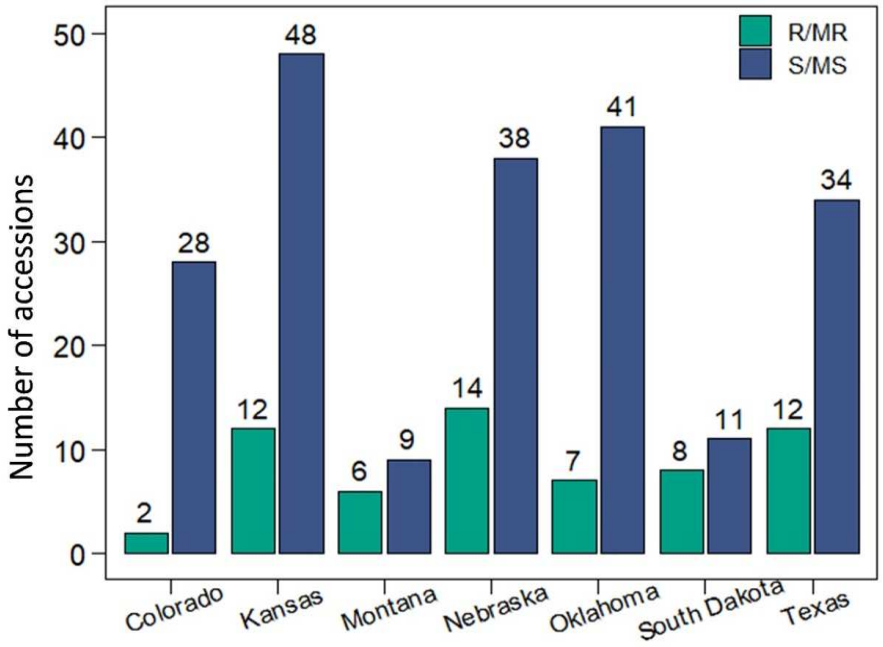

Figure 7. Distribution of the SNB resistance among the 274 accessions of hard winter wheat association mapping panel (HWWAMP) originating from different breeding

programs

In addition to SNB screening, we evaluated HWWAMP against three necrotrophic effectors (NEs), namely SnToxA, SnTox1, and SnTox3. The *P. nodorum* isolate Sn2000 contains two important NEs, SnToxA and SnTox1 (Liu & al., 2004); (Liu & al., 2006); (Friesen & al., 2006), that play a significant role in disease development. Out of the 274 accessions, 209 (76%), 15 (5%), and 65 (24%) were found sensitive to SnToxA, SnTox1, and SnTox3, respectively, indicating the higher prevalence of *Tsn1*-SnToxA and *Snn3*-SnTox3 interactions in the germplasm from the Great Plains region. Most of the accessions (76%) in our study were sensitive to SnToxA, owing to the presence of susceptibility gene *Tsn1* in most of the tested material. Sensitivity to SnToxA is regulated by the expression of sensitivity gene *Tsn1*, and happens to be the one with the largest positive effect on susceptibility (Faris & Zhang, 2010); (Liu Z. e., 2006). The frequency of SnToxA sensitive lines is varies in different germplasm; for example, only 10% of accessions were found sensitive in a British winter wheat germplasm collection (Downie & al., 2018), whereas it was 45% in Scandinavian varieties[30] and 65% in Western Australian spring wheat (Waters, Lichtenzveig, Rybak, & al., 2011). In contrast to SnToxA, 95% of HWWAMP lines (259) were insensitive to SnTox1, suggesting the absence of *Snn1* gene (Liu & al., 2004) in most of the accessions. Furthermore, we did not find any significant MTAs between SNPs and SnTox1 sensitivity, indicating a weak *Snn1*-SnTox1 interaction. A few accessions sensitive to SnTox1 in the current study are in line with previous reports in hexaploidy wheat (Ruud & al., 2019); (Downie & al., 2018); (Shi, 2015). The frequency of accessions sensitive to SnTox3 (24%) in the current

study was similar to that reported in the European germplasm (Friesen, Zhang, Solomon, Oliver, & Faris, 2008). We could not identify *Snn3* in GWAS analysis for SNB response due to lack of SnTox3 in Sn2000; however, GWAS for SnTox3 sensitivity identified a significant association in the region corresponding to *Snn3* gene suggesting the presence of *Snn3* in the winter wheat panel.

Further, we investigated whether NE sensitivity contributes toward the SNB susceptibility. We found a significant difference ($P < 2.2e-16$) for SNB severity between sensitive and insensitive groups for SnToxA. The insensitive lines were more resistant to SNB than the sensitive lines. No such differences were found between SnTox1 sensitive and insensitive groups (Fig. 1D). Interestingly, the highly resistant lines (score; 1-1.1), most were insensitive to all the three NEs (Table 2) and the highly susceptible lines (score; 4-5) were sensitive to at least one NE, suggesting the NE triggered susceptibility. A significant ($P < 0.001$) correlation was also observed between the effector sensitivity reaction and disease score in a recent study (Ruud & al., 2019). We also identified six lines that were insensitive to all three NEs (SnToxA, SnTox1, and SnTox3) but susceptible (Score; 3-5) to SNB isolate Sn2000 at the seedling stage, suggesting the possible interactions of other NE with host susceptibility gene(s) or lack of host resistance genes (Friesen & al., 2009). Necrotrophic effector-triggered susceptibility in the wheat-*P. nodorum* pathosystem is a complicated process and the effects can vary depending on the genetic backgrounds of the pathogen and host (Phan & al., 2018); (Peters-Haugrud, Zhang, Richards, & al., 2019).

Our GWAS identified significant associations on chromosomes 1B, 2AL, 2DS, 4AL,

5BL, 6BS, and 7AL, representing seven distinct QTLs for SNB resistance/susceptibility. Based on the types of markers used in similar studies (Adhikari, Jackson, Gurung, Hansen, & al., 2011); (Gurung, Mamidi, Bonman, Xiong, Brown-Guedira, & al., 2014); (Liu & al., 2015); (Tommasini, Schnurbusch, Fossati, Mascher, & al., 2007), it is difficult to precisely compare the previously identified regions to those of our study. However, to facilitate the comparison of QTLs reported in other studies with our study, we identified the approximate genomic locations of QTLs on IWGSC RefSeq ver 1.1 (IWGSC); et al., 2018). In agreement with the previous studies(Ruud & al., 2019); (Adhikari, Jackson, Gurung, Hansen, & al., 2011); (Gurung, Mamidi, Bonman, Xiong, Brown-Guedira, & al., 2014), the QTL with the largest effect was detected in the region corresponding to the genomic region of *Tsn1* (Gurung, Mamidi, Bonman, Xiong, Brown-Guedira, & al., 2014) . We identified one QTL (*QSnb.sdsu-2D*) on the short arm of chromosome 2D, which physically maps to 9 Mb on the wheat reference genome. Two recent studies (Ruud & al., 2019); (Liu & al., 2015) have also reported SNB resistance QTLs in the same region (~14-15 Mb) at the adult plant stage. This region also harbors the SnTox2 sensitivity gene *Snn2* (6-12 Mb) (Ruud & al., 2019); thus, *QSnb.sdsu-2D* identified in this study co-locates with this sensitivity gene.

A robust QTL (*QSnb.sdsu-7A*) was detected on the long arm of chromosome 7A, which physically mapped to the distal portion of the long arm at 721 Mb. Previous studies (Ruud & al., 2019); (Liu & al., 2015) have reported a QTL for seedling resistance on the long arm of chromosome 7A at around 550 Mb and 590 Mb. Therefore, *QSnb.sdsu-7A,* which maps 200 Mb distal, could be a novel QTL on the terminal end of chromosome

7A. Furthermore, we identified a significant association for SNB response at the seedling stage on the short arm of chromosome 6B, physically mapping around 30 Mb on the reference genome. Ruud et al (Ruud & al., 2019) recently reported an adult plant resistance QTL on the short arm of chromosome 6B, located at the same region (20-47 Mb) on the physical map. Thus, co-location of *QSnb.sdsu-6B* with Ruud et al (Ruud & al., 2019) suggests that the same locus may confer resistance at the juvenile and adult plant stage.

In addition to these regions, we identified significant QTLs on chromosomes 1B, 2AL, and 4AL. The QTL identified on chromosome 4AL was physically located at ~740 Mb on the reference genome. Liu et al, (Liu & al., 2015) also reported an association in a close approximation (~710 Mb) from a GWAS using some US winter wheat cultivars. Two other mapping studies (Liu & al., 2004) reported a significant QTL in the same region; however, we could not identify the physical location owing to different types of markers. Most likely, *QSnb.sdsu.4A* corresponds and validates these regions and plays a role in resistance/susceptibility at the seedling stage. The current study also validated another genomic region on chromosome 2AL at ~780 Mb. Several studies have identified loci for seedling and adult plant resistance in the same region (Downie, 2018) ; (Lin,2020). In a very recent report, Lin et al (Lin,2020) identified an adult plant resistance QTL in the same region positioned between 755 – 780 Mb, overlapping with the *QSnb.sdsu.2A*.

Further, we analyzed the effect of accumulation of resistant alleles at seven of the detected QTLs to verify the quantitative resistance. Six different genotype groups (group

0 to group 5) were observed carrying ‘zero’ to ‘six’ resistant alleles at identified loci, respectively. As found in earlier reports[41], accessions with a higher number of resistant alleles (either four or five) exhibited a high level of resistance. We compared the six groups using an FDR-corrected pairwise t-test and found significant differences in the level of resistance to SNB, which explains the additive and complex nature of SNB resistance (Friesen & Faris 2010); (Phan & al., 2018).In addition, we identified several released cultivars carrying four (‘Pioneer-2180’ and ‘Shocker’) or five (‘Colt’, ‘TAM304’, ‘Darrel’, ‘Hume’) resistance-associated alleles, with high resistance level. These accessions explain the effectiveness of pyramiding effector insensitivity and resistance-associated QTLs for SNB resistance.

Apart from GWAS for SNB response, we performed association analysis for sensitivity to SnToxA, SnTox1, and SnTox3. We identified significant MTAs for SnToxA and SnTox3; however, no association was detected for SnTox1. Liu et al, (2015) (Liu Z. e., 2015) also reported similar results from the GWA study employing winter wheat cultivars. The MTAs identified for SnToxA and SnTox3 corresponds to the genomic regions of *Tsn1*(Liu Z. e., 2006) and *Snn3* (Ruud, Windju, Belova, & al., 2017) genes, respectively. The low variation for SnTox1 sensitivity among HWWAMP accessions could be the potential reason for not detecting any MTAs for SnTox1 sensitivity.

The five genomic regions associated with SNB resistance/susceptibility were screened for candidate genes based on the using Chinese Spring reference genome RefSeq v1.1(International Wheat Genome Sequencing Consortium (IWGSC); et al., 2018). In wheat, majority of the characterized disease resistance genes encode intracellular immune

receptors of the nucleotide binding-site–leucine-rich repeat (NBS-LRR) family, wall-associated kinases (WAKs), receptor-like kinases (RLKs), and protein kinases as the protein product(Keller, Wicker, & Krattinger, 2018). For instance, *Tsn1*, encodes S/T protein kinase-NLR containing protein (Faris & Zhang, 2010). *Snn1* encodes a Wall-associated kinase protein (Shi, 2015). Similarly, *Stb6* governs resistance against *Septoria tritici* blotch in wheat and belongs to the wall-associated kinase family of proteins[58]. Therefore, these gene families are expected to play a role in the wheat disease defense response. Our study identified several genes encoding NBS-LRR domain, wall-associated kinases, receptor-like kinases, or protein kinases in the regions spanning identified QTLs (Table 4). These disease-related genes could be useful for the identification of potential candidates responsible for resistance/susceptibility to SNB.

In summary, we identified and validated several QTLs for SNB resistance/susceptibility in hard winter wheat. These QTLs could be easily employed in breeding programs using the associated markers to improve the SNB resistance in wheat. The comparison of groups carrying a different number of resistant/susceptibility alleles suggests the additive nature of SNB resistance. Thus, stacking of identified resistance-associated QTLs and known effector insensitivity genes could help in developing SNB resistant cultivars. The highly resistant winter wheat accessions (‘Pioneer-2180’ and ‘Shocker’) with up to five favorable alleles could be valuable germplasm for the wheat breeders. These accessions can be further evaluated against other prevalent isolates and for adult-plant resistance; and used in the breeding programs to improve SNB resistance.

3.5 Conclusion

The objective of this study was to evaluate the genetic basis of SNB resistance in wheat at the seedling stage and identify markers associated with SNB resistance. These markers could be used to improve the SNB resistance in wheat in an efficient way. In our study, we identified several winter wheats accessions with high resistance to SNB. These accessions are quite valuable for the wheat breeders as these could be directly used in the breeding programs to improve SNB resistance. Furthermore, we identified three novel QTLs, which could be easily employed in breeding programs using the associated markers. Additionally, we also validated three QTLs identified in different studies, which is very important for breeding purposes. The candidate genes analysis can play an important role in further characterization of the identified QTLs.

CHAPTER 4

GENERAL CONCLUSIONS AND RECOMMENDATIONS

The results from the study have unraveled some useful information on the sources of resistance to SNB disease. They will play an effective role in breeding programs against SNB which is an important fungal disease globally. In this study, we used a hard winter wheat association-mapping panel (HWWAMP), which turned out to be a good source of SNB resistant germplasm. The objectives of our study were to explore the resistant sources against SNB and perform GWAS to identify marker-trait associations for SNB resistance and sensitivity to SnToxA, SnTox1, and SnTox3.In this study; we identified 105 lines, which showed resistance or moderate-resistance reaction against SNB, indicating a good source of resistance present in the HWWAMP. Among all the resistant accessions identified in this study, only seven accessions exhibited a highly resistant reaction (score; 1-1.1) against the SNB. It was interesting to see that most of the breeding programs from where the entries were tested have developed a few highly resistant germplasms against SNB. It is important to note that, the susceptibility gene Tsn1 has retained in the hard winter wheat breeding programs of the central U.S. states, either because of the deliberate selection of resistance gene and/or it is linked to important agronomic traits such as hardiness.

Out of the 274 HWWAMP accessions, 209, 15, and 65 were found sensitive to SnToxA, SnTox1, and SnTox3, respectively, which indicates the higher activities of Tsn1-SnToxA and Snn3-SnTox3pathosystems. We identified the genomic regions harboring sensitivity

genesTsn1 and Snn3 in the association analysis. In this study, a majority of the accessions (76%) were sensitive to SnToxA, which may be due to the presence of susceptibly gene Tsn1 in most of the tested material. Our GWAS results for SnToxA infiltration also support the large proportion of our accessions being sensitive to SnToxA, as we identified a single genomic region on the long arm of chromosome 5B, corresponding to the genomic location of Tsn1. On the other hand, most of our lines (259) were insensitive against SnTox1, indicating the absence of Snn1 gene in most of the accessions of the HWWAMP. Snn1 is thought to be less frequent in North American hexaploidy wheat, compared to durum wheat germplasm. Furthermore, we did not find any significant marker-trait associations between SNPs and SnTox1 infiltration response, indicating a weak Snn1-SnTox1 interaction. A modest number of our tested germplasm (24%) showed a sensitive reaction towards SnTox3, which could be due to the presence of Snn3 gene in some of our accessions. Our GWAS results for the SnTox3 infiltration response further support this, where we identified a significant association on the short arm of chromosome 5B, which harbors the Snn3 gene. The frequency of sensitive accessions toSnTox3 (24%) in this study was similar to that reported in European germplasm.

In this study, we also investigated whether NEs sensitivity affects the Sn2000 driven disease development. In terms of SnToxA, we found a significant difference between sensitive and insensitive groups in the contribution to SNB, with insensitive lines being more resistant to SNB, compared to the sensitive genotypes. However, we did not find any significant relationship in the case of SnTox1 and SnTox3. Out of the seven highly

resistant lines (score; 1-1.1), 11 were insensitive towards all three NEs, while most of the highly susceptible lines (score; 4-5) had at least one NE initiated sensitivity, which suggests the necrotrophic effector-triggered susceptibility. A significant ($p < 0.0001$) correlation was also observed between the effector sensitivity reaction and disease score. Furthermore, we identified six lines which were insensitive against all three NEs (SnToxA, SnTox1, and SnTox3) but susceptible (Score; 3-5) to SNB isolate Sn2000 at the seedling stage, suggesting the possible interactions of other NE-host susceptibility gene or due to the lack of host resistance genes.

Furthermore, we used GWAS to identify genomic regions controlling resistance to SNB and sensitivity to SnToxA, SnTox1, and SnTox3. For SNB resistance, we identified significant associations on chromosomes 1B, 2BL, 2DS, 4AL, 5BL, 6BS, and 7AL, representing seven distinct QTLs. The QTL with the largest effect was detected on the long arm of chromosome 5B. This region corresponds to the genomic region of Tsn1, responsible for sensitivity to SnToxA. Another significant QTL was detected on the long arm of chromosome 7A, which physically mapped to the distal portion of the long arm at 721 Mb. This suggests that this could be a novel QTL also conferring resistance against SNB. Furthermore, we identified a significant association on the short arm of chromosome 2B, physically mapping to 58 Mb. We did not find any study reporting a QTL in this region for seedling resistance against SNB; however, Ruud et al, (2017) reported a QTL in the same region (~68 Mb) for adult plant resistance to SNB using different isolates. Therefore, this might be a potential novel QTL controlling seedling

resistance. An additional novel QTL was identified on chromosome 1BL, physically located on the long arm at ~620 Mb. Several studies have reported a QTL on short arm of 1B corresponding to Snn1; however, we did not find any reports for the long arm of chromosome 1B.

In addition to these regions, we identified significant QTLs on chromosomes 4AL, 6BL, and 2AL. The QTL identified on chromosome 4AL was physically located at ~740 Mb. Similarly, we detected a QTL on the long arm of chromosome 6B, mapped at ~710 Mb. Lastly, we identified a QTL at ~770 Mb on the long arm of chromosome 2B.

Apart from GWAS for SNB resistance, we also performed association analysis for sensitivity to SnToxA, SnTox1, and SnTox3. We identified significant marker-trait associations for SnToxA and SnTox3; however, no association was detected in the case of SnTox1. We identified a single genomic region was on the long arm of chromosome 5B for SnToxA, which corresponds to the genomic region of Tsn1. Similarly, we identified a significant association for SnTox3 sensitivity on the small arm of chromosome 5B, which corresponds to the genomic location of Snn3. The low variation for SnTox1 sensitivity among HWWAMP accessions could be the potential reason for not detecting any marker-trait associations for SnTox1 sensitivity. Also, the frequency of Snn1 in wheat germplasm from the US region has been reported to be less as compared with other genes.

The six genomic regions associated with SNB resistance (except for the 5BL region) were screened for underlying candidate genes based on the CS reference genome 1.1. In

wheat, numerous genes associated with resistance to different diseases have been fully or partially characterized. The majority of these characterized genes encode intracellular immune receptors of the nucleotide binding-site–leucine-rich repeat (NBS-LRR) family, wall-associated kinases (WAKs), receptor-like kinases (RLKs), and protein kinases as the protein product. For instance, Tsn1, which is associated with resistance against SNB and Tan spot of wheat, encodes S/T protein kinase-NLR containing protein. Snn1, also associated with SNB encodes a Wall-associated kinase protein56. Similarly, Stb6 governs resistance against Septoria tritici blotch in wheat and belongs to the wall-associated kinase family of proteins61. Therefore, these gene families are expected to play a role in the wheat disease defense system. In our study, we identified several genes in the six genomic regions encoding NBS-LRR domain, wall-associated kinases, receptor-like kinases, or protein kinases. These disease-related genes could be useful for the identification of potential candidates underlying the identified QTLs.

The objective of this study was to evaluate the genetic basis of SNB resistance in wheat at the seedling stage and identify markers associated with SNB resistance. These markers could be used to improve the SNB resistance in wheat in an efficient way. In our study, we identified several winter wheats accessions with high resistance to SNB. These accessions are quite valuable for the wheat breeders as these could be directly used in the breeding programs to improve SNB resistance. Furthermore, we identified three novel QTLs, which could be easily employed in breeding programs using the associated markers. Additionally, we also validated three QTLs identified in different studies, which

is also very important for breeding purposes. The candidate genes analysis can play an important role in further characterization of the identified QTLs.

References

FAOSTAT. Available at: http://www.fao.org/faostat/en/#data/QC. (Accessed: 22nd May 2020)

Tester, M. & Langridge, P. Breeding technologies to increase crop production in a changing world. Science 327, 818–822 (2010).

Figueroa, M., Hammond-Kosack, K. E. & Solomon, P. S. A review of wheat diseases-a field perspective. Mol. Plant Pathol. 19, 1523–1536 (2018).

Oliver, R. P., Tan, K. C. & Moffat, C. S. Necrotrophic Pathogens of Wheat. in Encyclopedia of Food Grains: Second Edition 4–4, 273–278 (Elsevier Inc., 2015).

Quaedvlieg, W. et al. Sizing up septoria. Stud. Mycol. 75, 307–390 (2013).

Francki, M. G. Improving Stagonospora nodorum resistance in wheat: A review. Crop Science 53, 355–365 (2013).

Cowger, C., Ward, B., Brown-Guedira, G. & Brown, J. K. M. Role of Effector-Sensitivity Gene Interactions and Durability of Quantitative Resistance to Septoria Nodorum Blotch in Eastern U.S. Wheat. Front. Plant Sci. 11, 155 (2020).

Bhathal, J. S., Loughman, R. & Speijers, J. Yield reduction in wheat in relation to leaf disease from yellow (tan) spot and septoria nodorum blotch. Eur. J. Plant Pathol. 109, 435–443 (2003).

Blixt, E., Djurle, A., Yuen, J. & Olson, Å. Fungicide sensitivity in Swedish isolates of Phaeosphaeria nodorum. Plant Pathol. 58, 655–664 (2009).

Pereira, D. A., McDonald, B. A. & Brunner, P. C. Mutations in the CYP51 gene reduce DMI sensitivity in Parastagonospora nodorum populations in Europe and China. Pest Manag. Sci. 73, 1503–1510 (2017).

Jones, J. D. G. & Dangl, J. L. The plant immune system. Nature 444, 323–329 (2006).

HH Flor. The complementary genic systems in flax and flax rust. Adv. Genet. 8, 29–54 (1956).

Friesen, T. L., Meinhardt, S. W. & Faris, J. D. The Stagonospora nodorum-wheat pathosystem involves multiple proteinaceous host-selective toxins and corresponding host sensitivity genes that interact in an inverse gene-for-gene manner. Plant J. 51, 681–692 (2007).

Friesen, T. L. & Faris, J. D. Characterization of the wheat- Stagonospora nodorum disease system: what is the molecular basis of this quantitative necrotrophic disease interaction? †. Can. J. Plant Pathol. 32, 20–28 (2010).

Ballance, G., Lamari, L. & Bernier, C. Purification and characterization of a host-selective necrosis toxin from Pyrenophora tritici-repentis. Physiol. Mol. Plant Pathol. 35, 203–213 (1989).

Faris, J. D. et al. A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. Proc. Natl. Acad. Sci. U. S. A. 107, 13544–13549 (2010).

Liu, Z. et al. The Tsn1-ToxA interaction in the wheat-Stagonospora nodorum pathosystem parallels that of the wheat-tan spot system. Genome 49, 1265–1273 (2006).

Friesen, T. L. et al. Emergence of a new disease as a result of interspecific virulence gene transfer. Nat. Genet. 38, 953–956 (2006).

Lu, S., Gillian Turgeon, B. & Edwards, M. C. A ToxA-like protein from Cochliobolus heterostrophus induces light-dependent leaf necrosis and acts as a virulence factor with host selectivity on maize. Fungal Genet. Biol. 81, 12–24 (2015).

Friesen, T. L., Holmes, D. J., Bowden, R. L. & Faris, J. D. ToxA Is Present in the U.S. Bipolaris sorokiniana Population and Is a Significant Virulence Factor on Wheat Harboring Tsn1. Plant Dis. 102, 2446–2452 (2018).

Abeysekara, N. S., Friesen, T. L., Keller, B. & Faris, J. D. Identification and characterization of a novel host-toxin interaction in the wheat-Stagonospora nodorum pathosystem. Theor. Appl. Genet. 120, 117–126 (2009).

Friesen, T. L., Zhang, Z., Solomon, P. S., Oliver, R. P. & Faris, J. D. Characterization of the interaction of a novel Stagonospora nodorum host-selective toxin with a wheat susceptibility gene. Plant Physiol. 146, 682–693 (2008).

Friesen, T. l., Chu, C., Xu, S. S. & Faris, J. D. SnTox5-Snn5: A novel Stagonospora nodorum effector-wheat gene interaction and its relationship with the SnToxA-

Tsn1 and SnTox3-Snn3-B1 interactions. Mol. Plant Pathol. 13, 1101–1109 (2012).

Gao, Y. et al. Identification and Characterization of the SnTox6- Snn6 Interaction in the Parastagonospora nodorum –Wheat Pathosystem. Mol. Plant-Microbe Interact. 28, 615–625 (2015).

Shi, G. et al. The Wheat Snn7 Gene Confers Susceptibility on Recognition of the Parastagonospora nodorum Necrotrophic Effector SnTox7. Plant Genome 8, plantgenome2015.02.0007 (2015).

Zhang, Z. et al. Two putatively homoeologous wheat genes mediate recognition of SnTox3 to confer effector-triggered susceptibility to Stagonospora nodorum. Plant J. 65, 27–38 (2011).

Arseniuk, E. et al. QTL controlling partial resistance to Stagonospora nodorum leaf blotch in winter wheat cultivar Alba. Euphytica 137, 225–231 (2004).

Czembor, P. C. et al. QTL mapping of partial resistance in winter wheat to Stagonospora nodorum blotch. Genome 46, 546–554 (2003).

Friesen, T. L. et al. Host-selective toxins produced by Stagonospora nodorum confer disease susceptibility in adult wheat plants under field conditions. Theor. Appl. Genet. 118, 1489–1497 (2009).

Liu, Z. H. et al. Genetic and physical mapping of a gene conditioning sensitivity in wheat to a partially purified host-selective toxin produced by Stagonospora nodorum. Phytopathology 94, 1056–1060 (2004).

Ruud, A. K., Windju, S., Belova, T., Friesen, T. L. & Lillemo, M. Mapping of SnTox3–Snn3 as a major determinant of field susceptibility to Septoria nodorum leaf blotch in the SHA3/CBRD × Naxos population. Theor. Appl. Genet. 130, 1361–1374 (2017).

Sidhu, J. S. et al. Genome-Wide Association Study Uncovers Novel Genomic Regions Associated With Coleoptile Length in Hard Winter Wheat. Front. Genet. 10, 1345 (2020).

Gupta, P. K., Kulwal, P. L. & Jaiswal, V. Association mapping in crop plants: Opportunities and challenges. in Advances in Genetics 85, 109–147 (Academic Press Inc., 2014).

Ruud, A. K. et al. Genome-Wide Association Mapping of Resistance to Septoria Nodorum Leaf Blotch in a Nordic Spring Wheat Collection. Plant Genome 12, 180105 (2019).

Korte, A. & Farlow, A. The advantages and limitations of trait analysis with GWAS: a review. Plant Methods 9, 29 (2013).

Ayana, G. T. et al. Genome-wide association study for spot blotch resistance in hard winter wheat. Front. Plant Sci. 9, (2018).

Halder, J. et al. Mining and genomic characterization of resistance to tan spot, Stagonospora nodorum blotch (SNB), and Fusarium head blight in Watkins core collection of wheat landraces. BMC Plant Biol. 19, 1–15 (2019).

Sukumaran, S., Dreisigacker, S., Lopes, M., Chavez, P. & Reynolds, M. P. Genome-wide association study for grain yield and related traits in an elite spring wheat population grown in temperate irrigated environments. Theor. Appl. Genet. 128, 353–363 (2014).

Adhikari, T. B., Jackson, E. W., Gurung, S., Hansen, J. M. & Bonman, J. M. Association mapping of quantitative resistance to Phaeosphaeria nodorum in spring wheat landraces from the USDA national small grains collection. Phytopathology 101, 1301–1310 (2011).

Gurung, S. et al. Genome-Wide Association Study Reveals Novel Quantitative Trait Loci Associated with Resistance to Multiple Leaf Spot Diseases of Spring Wheat. PLoS One 9, e108179 (2014).

Liu, Z. et al. Evaluation and Association Mapping of Resistance to Tan Spot and Stagonospora Nodorum Blotch in Adapted Winter Wheat Germplasm. Plant Dis. 99, 1333–1341 (2015).

Phan, H. T. T. et al. Novel sources of resistance to Septoria nodorum blotch in the Vavilov wheat collection identified by genome-wide association studies. Theor. Appl. Genet. 131, 1223–1238 (2018).

Tommasini, L., Schnurbusch, T., Fossati, D., Mascher, F. & Keller, B. Association mapping of Stagonospora nodorum blotch resistance in modern European winter wheat varieties. Theor. Appl. Genet. 115, 697–708 (2007).

Guttieri, M. J. et al. Variation for grain mineral concentration in a diversity panel of current and historical Great Plains hard winter wheat germplasm. Crop Sci. 55, 1035–1052 (2015).

Ramakrishnan, S. M., Sidhu, J. S. & Ali, S. Molecular characterization of bacterial leaf streak resistance in hard winter wheat. (2019). doi:10.7717/peerj.7276

Xu, S. S., Friesen, T. L. & Mujeeb-Kazi, A. Seedling resistance to tan spot and Stagonospora nodorum blotch in synthetic hexaploid wheats. in Crop Science 44, 2238–2245 (2004).

Oliver, R. E., Cai, X., Wang, R.-C., Xu, S. S. & Friesen, T. L. Resistance to Tan Spot and Stagonospora nodorum Blotch in Wheat-Alien Species Derivatives. Plant Dis. 92, 150–157 (2008).

Mergoum, M. et al. Reaction of elite wheat genotypes from the northern Great Plains of North America to septoria diseases. Plant Dis. 91, 1310–1315 (2007).

Peters Haugrud, A. R., Zhang, Z., Richards, J. K., Friesen, T. L. & Faris, J. D. Genetics of variable disease expression conferred by inverse gene-for-gene interactions in the wheat-parastagonospora nodorum pathosystem. Plant Physiol. 180, 420–434 (2019).

Liu, Z. et al. The cysteine rich necrotrophic effector SnTox1 produced by Stagonospora nodorum triggers susceptibility of wheat lines harboring Snn1. PLoS Pathog. 8, e1002467–e1002467 (2012).

Reddy, L., Friesen, T. L., Meinhardt, S. W., Chao, S. & Faris, J. D. Genomic Analysis of the Snn1 Locus on Wheat Chromosome Arm 1BS and the Identification of Candidate Genes. Plant Genome 1, 55–66 (2008).

Liu, Z. et al. SnTox3 Acts in Effector Triggered Susceptibility to Induce Disease on Wheat Carrying the Snn3 Gene. PLoS Pathog. 5, e1000581 (2009).

Winterberg, B. et al. The necrotrophic effector protein SnTox3 re-programs metabolism and elicits a strong defence response in susceptible wheat leaves. BMC Plant Biol. 14, (2014).

Oliver, R. P., Friesen, T. L., Faris, J. D. & Solomon, P. S. Stagonospora nodorum : From Pathology to Genomics and Host Resistance . Annu. Rev. Phytopathol. 50, 23–43 (2012).

Downie, R. C. et al. Assessing European Wheat Sensitivities to Parastagonospora nodorum Necrotrophic Effectors and Fine-Mapping the Snn3-B1 Locus Conferring Sensitivity to the Effector SnTox3. Front. Plant Sci. 9, 881 (2018).

Waters, O. D. C., Lichtenzveig, J., Rybak, K., Friesen, T. L. & Oliver, R. P. Prevalence and importance of sensitivity to the Stagonospora nodorum necrotrophic effector

SnTox3 in current Western Australian wheat cultivars. Crop Pasture Sci. 62, 556–562 (2011).

Shi, G. et al. The hijacking of a receptor kinase–driven pathway by a wheat fungal pathogen leads to disease. Sci. Adv. 2, e1600822 (2016).

Oliver, R. P., Rybak, K., Solomon, P. S. & Ferguson-Hunt, M. Prevalence of ToxA-sensitive alleles of the wheat gene Tsn1 in Australian and Chinese wheat cultivars. Crop Pasture Sci. 60, 348 (2009).

International Wheat Genome Sequencing Consortium (IWGSC), T. I. W. G. S. C. et al. Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science 361, eaar7191 (2018).

Lin, M. et al. Genetic mapping using a wheat multi-founder population reveals a locus on chromosome 2A controlling resistance to both leaf and glume blotch caused by the necrotrophic fungal pathogen Parastagonospora nodorum. Theor. Appl. Genet. 133, 785–808 (2020).

Lin, M. et al. Identification and cross-validation of genetic loci conferring resistance to Septoria nodorum blotch using a German multi-founder winter wheat population. Theor. Appl. Genet. 1, 3 (2020).

Francki, M. G., Walker, E., McMullan, C. J. & Morris, W. G. Multi-Location Evaluation of Global Wheat Lines Reveal Multiple QTL for Adult Plant Resistance to

Septoria Nodorum Blotch (SNB) Detected in Specific Environments and in Response to Different Isolates. Front. Plant Sci. 11, (2020).

Liu, Z. H. et al. Quantitative trait loci analysis and mapping of seedling resistance to Stagonospora nodorum leaf blotch in wheat. Phytopathology 94, 1061–1067 (2004).

Keller, B., Wicker, T. & Krattinger, S. G. Advances in Wheat and Pathogen Genomics: Implications for Disease Control. Annu. Rev. Phytopathol. 56, 67–87 (2018).

Saintenac, C. et al. Wheat receptor-kinase-like protein Stb6 controls gene-for-gene resistance to fungal pathogen Zymoseptoria tritici. Nat. Genet. 50, 368–374 (2018).

Abdullah, S. et al. Characterization of Pyrenophora tritici-repentis (Tan spot of wheat) races in Baltic states and Romania. Plant Pathol. J. 33, 133–139 (2017).

Faris, J., Anderson, J., Francl, L. & Jordahl, J. Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from Pyrenophora tritici-repentis. Phytopathology 86, 459–463 (1996).

Team, R. C. R: A language and environment for statistical computing. (2014).

Wu, J. Minique: An R package for linear mixed model analyses. R Found. Stat. Comput. Vienna (2014).

Cavanagh, C. R. et al. Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proc. Natl. Acad. Sci. (2013). doi:10.1073/pnas.1217133110

Wang, S. et al. Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. Plant Biotechnol. J. 12, 787–796 (2014).

Pritchard, J. K., Stephens, M. & Donnelly, P. Inference of Population Structure Using Multilocus Genotype Data. Genetics 155, 945 LP – 959 (2000).

Earl, D. A. & vonHoldt, B. M. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4, 359–361 (2012).

Evanno, G., Regnaut, S. & Goudet, J. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Mol. Ecol. 14, 2611–2620 (2005).

Ramasamy, R. K., Ramasamy, S., Bindroo, B. B. & Naik, V. G. STRUCTURE PLOT: A program for drawing elegant STRUCTURE bar plots in user friendly interface. Springerplus 3, 1–3 (2014).

Bradbury, P. J. et al. TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23, 2633–2635 (2007).

Yu, J. et al. A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat. Genet. 38, 203–208 (2006).

Liu, X., Huang, M., Fan, B., Buckler, E. S. & Zhang, Z. Iterative Usage of Fixed and Random Effect Models for Powerful and Efficient Genome-Wide Association Studies. PLOS Genet. 12, e1005767 (2016).

Tang, Y. et al. GAPIT Version 2: An Enhanced Integrated Tool for Genomic Association and Prediction. Plant Genome 9, plantgenome2015.11.0120 (2016).

Endelman, J. B. & Jannink, J.-L. Shrinkage Estimation of the Realized Relationship Matrix. G3: Genes|Genomes|Genetics 2, 1405–1413 (2012).

Benjamini, Y. & Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. Ser. B 57, 289–300 (1995).

Appendix Table 1. Grand mean for SNB responses (0-5 scale) and Necrotrophic effectors (NEs) reactions evaluated on 274 accessions of hard winter wheat association mapping panel (HWWAMP). The NEs sensitivity was recorded as I (Insensitive) or S (Sensitive) reaction.

Accession	SNB Response	SnToxA Reaction	SnTox1 Reaction	SnTox3 Reaction
2180	1	I	I	I
2145	2.777777778	S	I	S
2174-05	3.222222222	S	I	I
ABOVE	2.333333333	S	I	I
AKRON	3.333333333	S	S	I
ALICE	1.888888889	I	I	S
ALLIANCE	1.333333333	I	I	I
ANTELOPE	3.555555556	S	I	I
ANTON	1.222222222	S	I	S
ARAPAHOE	3.777777778	S	I	I
ARLIN	2.222222222	I	I	S
AVALANCHE	2	I	I	S
BAKERS_WHITE	2	S	I	I
BENNETT	2.444444444	I	I	I
BIG_SKY	2.777777778	I	I	I
BILL_BROWN	3.111111111	I	I	S
BILLINGS	1.444444444	I	I	I
BISON	2.333333333	S	I	S
BOND_CL	3	S	S	S
BUCKSKIN	3.333333333	S	I	I
BURCHETT	4	S	I	I
BYRD	2.222222222	S	I	I
CAMELOT	2	I	I	S
CAPROCK	2.777777778	S	I	I
CARSON	3	S	I	I
CENTERFIELD	3.111111111	S	I	I
CENTURK78	2.555555556	S	I	I
CENTURY	1.333333333	I	I	S
CHENEY	4.777777778	S	I	I
CHEYENNE	3.888888889	S	I	I

CHISHOLM	4	S	I	S
CO03064	2.444444444	S	I	I
CO03W043	2.333333333	S	S	S
CO04025	4	S	I	I
CO04393	3.333333333	I	I	S
CO04499	3.444444444	S	I	S
CO07W245	3.111111111	S	I	S
CO940610	2.777777778	S	I	I
COLT	1	I	I	S
COMANCHE	3.666666667	S	I	I
COSSACK	3	I	I	I
COUGAR	3.555555556	S	I	I
CREST	2.555555556	S	I	I
CRIMSON	2	I	I	S
CULVER	4.111111111	S	I	I
CUSTER	4	S	I	I
CUTTER	2	S	S	I
DANBY	3	S	I	I
DARRELL	1.555555556	I	I	I
DAWN	4.111111111	S	I	I
DECADE	1.333333333	I	I	I
DENALI	3.666666667	S	I	I
DODGE	3.666666667	S	I	I
DUSTER	4	S	II	I
E2041	1	I	I	I
EAGLE	4.444444444	S	I	I
ENHANCER	3.777777778	S	I	I
EXPEDITION	4	S	I	I
FULLER	3.444444444	S	I	I
G1878	3.222222222	S	I	I
GALLAGHER	3.555555556	S	I	I
GARRISON	2	S	I	I
GENOU	1.111111111	I	I	I
GENT	3.111111111	S	I	I
GOODSTREAK	4.222222222	S	I	I
GUYMON	3.222222222	S	I	I
HAIL	4	S	S	I
HALLAM	4	S	I	I
HALT	2.666666667	S	S	S

HARDING	4	S	I	I
HARRY	3.333333333	S	I	I
HATCHER	2.333333333	S	I	I
HEYNE	3.555555556	S	I	S
HG-9	4	S	I	I
HOMESTEAD	3.888888889	S	I	I
HONDO	3.333333333	S	I	S
HUME	1.666666667	I	I	I
HV906-865	2.666666667	S	I	I
HV9W03-1379R	3.333333333	S	I	S
HV9W03-1551WP	2.333333333	I	I	I
HV9W03-1596R	1.333333333	I	I	I
HV9W05-1280R	2	S	I	I
INFINITY_CL	3	S	I	S
INTRADA	2.333333333	S	I	I
JAGALENE	3.888888889	S	I	S
JAGGER	2	S	I	I
JERRY	4.666666667	S	I	I
JUDEE	4.222222222	S	I	S
JUDITH	3.333333333	S	I	I
JULES	4	S	S	I
KARL_92	3.222222222	S	I	I
KAW61	4	S	I	I
KEOTA	3.333333333	S	I	I
KHARKOF	3.888888889	S	S	I
KIOWA	4.222222222	S	I	I
KIRWIN	3.666666667	S	I	I
KS00F5-20-3	2	S	I	S
LAKIN	3.888888889	S	I	S
LAMAR	3.111111111	S	I	I
LANCER	3.777777778	S	I	I
LARNED	4.111111111	S	I	I
LINDON	3.444444444	S	I	I
LONGHORN	4.444444444	S	I	I
MACE	2	S	I	S
MCGILL	3.777777778	S	I	I
MILLENNIUM	1.888888889	I	I	I
MIT	1.666666667	S	I	I
MT0495	2	S	I	I

MT06103	1.777777778	I	I	I
MT85200	2.888888889	S	I	I
MT9904	1.666666667	I	I	I
MT9982	3.555555556	S	I	I
NE02558	2	S	I	I
NE04490	2	S	I	I
NE05430	2.555555556	S	I	I
NE05496	2.333333333	I	I	I
NE05548	2.666666667	S	I	I
NE06545	1.222222222	I	I	I
NE06607	2.777777778	S	I	I
NE99495	3.333333333	I	I	S
NEKOTA	1.111111111	I	I	I
NELL	1.222222222	I	I	I
NEOSHO	2.222222222	S	I	I
NEWTON	3.666666667	S	I	I
NI06736	3	S	I	I
NI06737	2.111111111	S	I	I
NI07703	3.444444444	S	I	I
NI08707	2.888888889	S	I	I
NI08708	2	S	I	S
NIOBRARA	4	S	I	I
NORKAN	4.333333333	S	I	I
NORRIS	3.444444444	S	I	I
NUFRONTIER	1.555555556	I	I	N
NUHORIZON	1.888888889	I	I	S
NUPLAINS	3.222222222	S	I	I
NUSKY	2	S	I	I
NW03666	1.444444444	I	I	I
OGALLALA	3	S	I	I
OK_RISING	4	S	S	I
OK02405	3.888888889	S	I	S
OK04111	4	S	I	I
OK04415	3.333333333	S	I	I
OK04507	2.222222222	S	I	I
OK04525	3.333333333	S	S	I
OK05511	2.555555556	S	I	S
OK05134	4	S	I	I
OK05204	3.444444444	S	I	S

OK05303	3.888888889	S	I	I
OK05312	2.222222222	S	I	I
OK05526	3	S	I	I
OK05711W	3.111111111	S	I	I
OK05723W	1.111111111	I	S	S
OK05830	2.777777778	I	I	I
OK06114	2.111111111	S	S	I
OK06210	2.777777778	I	I	I
OK06318	3.222222222	S	I	I
OK06319	2.222222222	S	I	S
OK06336	4	S	I	S
OK07231	3.555555556	I	I	I
OK07S117	2	I	S	S
OK08328	2.555555556	I	I	I
OK09634	1	I	I	I
OK101	1.333333333	I	I	I
OK10119	2.444444444	S	I	I
OK102	4	S	I	I
OK1067071	3.333333333	S	I	S
OK1067274	3	I	I	I
OK1068002	4	S	I	I
OK1068009	3.444444444	S	I	I
OK1068026	4	S	I	I
OK1068112	3.111111111	S	I	I
OK1070267	3.111111111	I	I	S
OK1070275	2.555555556	S	I	S
ONAGA	2.111111111	S	I	I
OVERLAND	3.444444444	S	I	I
OVERLEY	2.222222222	S	I	I
PARKER	4.333333333	S	I	I
PARKER76	4.888888889	S	I	I
PETE	4	S	I	I
PLATTE	3.555555556	S	I	S
POSTROCK	2.444444444	S	I	I
PRAIRIE_RED	3.222222222	S	I	S
PRONGHORN	4.555555556	S	I	I
PROWERS	3.222222222	S	I	I
RAWHIDE	4	S	I	I
REDLAND	4	S	I	I

RIPPER	3.111111111	S	I	S
RITA	2.888888889	S	S	S
ROBIDOUX	3.555555556	S	I	I
RONL	3.555555556	S	I	I
ROSE	2	I	I	I
ROSEBUD	NA+B278:D280	S	I	I
SAGE	4.444444444	S	I	I
SANDY	3.222222222	S	I	I
SANTA_FE	2.777777778	S	I	I
SCOUT66	3.666666667	S	S	I
SD00111-9	3	S	I	I
SD01058	3.222222222	S	I	I
SD01237	3.222222222	S	I	I
SD05118	3.444444444	I	I	I
SD05210	1	I	I	I
SD05W018	4.111111111	S	I	I
SETTLER_CL	3	S	I	I
SHAWNEE	4	I	I	I
SHOCKER	1.444444444	I	I	I
SIOUXLAND	2.111111111	S	I	I
SMOKYHILL	1.888888889	S	I	I
SPARTAN	4.111111111	S	I	I
STANTON	3	I	I	S
STURDY	3.222222222	S	I	I
STURDY_2K	1	I	I	I
TAM105	3.111111111	S	I	I
TAM107	4	S	I	I
TAM109	2.333333333	S	I	I
TAM110	4	S	I	S
TAM111	3.333333333	S	I	S
TAM112	3.444444444	S	I	I
TAM202	2.222222222	S	I	I
TAM203	1.222222222	S	I	I
TAM303	3.111111111	S	I	S
TAM304	1	I	I	I
TAM400	1.777777778	S	N	N
TAM401	1.666666667	I	I	I
TAMW-101	2.555555556	S	I	I
TANDEM	1.222222222	I	I	I

TARKIO	3.555555556	S	I	I
TASCOSA	4	I	I	S
THUNDER_CL	3.333333333	S	I	S
THUNDERBOLT	3	S	I	S
TREGO	2	I	I	I
TRISON	3.333333333	S	I	I
TRIUMPH64	2.333333333	I	I	S
TURKEY_NEBSEL	2.444444444	S	I	I
TX00V1131	1.666666667	S	I	I
TX01A5936	2.444444444	S	I	I
TX01M5009-28	1.222222222	I	I	I
TX01V5134RC-3	3.444444444	S	I	I
TX02A0252	4	S	I	I
TX03A0148	4	S	I	S
TX03A0563	3.111111111	S	I	S
TX04A001246	2	I	I	S
TX04M410211	1.777777778	S	I	I
TX04V075080	3	S	I	I
TX05A001188	4	S	I	I
TX05A001822	3.666666667	S	I	I
TX05V7259	4.444444444	S	I	S
TX05V7269	4.666666667	S	I	I
TX06A001132	4.444444444	S	I	I
TX06A001281	3	S	I	I
TX06A001386	4	S	I	I
TX06V7266	4	I	I	I
TX07A001279	1.555555556	I	I	S
TX07A001318	4.111111111	S	I	S
TX07A001420	3.666666667	S	I	S
TX86A5606	5	S	I	I
TX86A6880	3	S	I	I
TX86A8072	3.555555556	S	I	S
TX96D1073	1.888888889	S	I	S
TX99A0153-1	3.333333333	S	I	I
TX99U8618	2.444444444	I	I	I
VENANGO	3.222222222	S	I	S
VISTA	4	S	I	I
VONA	3.444444444	S	I	I
W04-417	2.888888889	S	I	S

WAHOO	3.888888889	S	I	I
WARRIOR	3.666666667	S	I	I
WB411W	3.222222222	I	I	I
WENDY	3.444444444	S	I	S
WESLEY	1.222222222	I	I	I
WICHITA	4.222222222	S	I	I
WINDSTAR	1.222222222	I	I	I
WINOKA	2.222222222	S	I	I
YELLOWSTONE	4.222222222	S	I	I
YUMAR	3	S	I	I